UTILIZATION OF PLANT INULIN

IN YOUNG RUMINANTS

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A Thesis Submitted in Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy in Animal Production Technology

Suranaree University of Technology

Academic Year 2017

การใช้ประโยชน์ของอินนูลินจากพืชในสัตว์เคี้ยวเอื้องวัยอ่อน

นางสาวกนกวรรณ ขำขจร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต

สาขาวิชาเทคโนโลยีการผลิตสัตว์

มหาวิทยาลัยเทคโนโลยีสุรนารี

ปีการศึกษา 2560

UTILIZATION OF PLANT INULIN IN YOUNG RUMINANTS

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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กนกวรรณ ขำขจร : การใช้ประโยชน์ของอินนูลินจากพืชในสัตว์เคี้ยวเอื้องวัยอ่อน (UTILIZATION OF PLANT INULIN IN YOUNG RUMINANTS) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.ปราโมทย์ แพงคำ, 135 หน้า.

วัตถุประสงค์ของการศึกษาครั้งนี้เพื่อศึกษาศักยภาพของอินนูลินจากพืช ได้แก่ ชิโคลี (*Cichorium intybus* L.) และแก่นตะวัน (*Helianthus tuberosus* L.) ในสัตว์เคี้ยวเอื้องวัยอ่อน

การทดลองที่ 1 ได้ทำการทดลองโดยใช้อินนูลินจากชิโคลีระดับต่าง ๆ ต่อปริมาณของแบคทีเรียโดยใช้ตัวอย่างมูลสดของลูกโคสายพันธุ์เจอร์ซี่โดยทำการบ่มในหลอดทดลอง ซึ่งมีทั้งหมด 2 ปัจจัยได้แก่ แป้ง 2 ระดับ (0 และ 1% ตามลำดับ) และปัจจัยจากระดับของอินนูลินจากชิโคลี 4 ระดับ (0 1 2 และ 4% ตามลำดับ) โดยวางแผนการทดลองแบบ 2x4 factorial arrangement in CRD ในการเพาะเลี้ยงเชื้อจะประกอบไปด้วยอาหารเลี้ยงเชื้อที่ผ่านการฆ่าเชื้อ + สารละลายจากมูลโคสด + อินนูลินจากชิโคลีระดับต่างๆ และบ่มที่ 37 องศาเซลเซียส เป็นระยะเวลา 24 และ 48 ชั่วโมงในตู้บ่มที่มีการเขย่าตลอดเวลา ในการศึกษานี้พบว่า การเพิ่มขึ้นของผลผลิตก๊าซทั้งหมดจากการหมักด้วยมูลกับอินนูลินที่ 24 ชั่วโมงมีอัตราการเพิ่มขึ้นเป็นเส้นตรง ในขณะเดียวกันเมื่อเพิ่มระดับของอินนูลินก็ส่งผลทำให้ปริมาณผลผลิตก๊าซสูงขึ้นตามไปด้วย และยังพบว่า ผลผลิตก๊าซเพิ่มขึ้นเมื่อใช้เวลาในการบ่มเพิ่มขึ้นจาก 0 ถึง 24 ชั่วโมง ในทางตรงกันข้ามค่าความเป็นกรด-ด่างของการหมักมูลโคที่ 24 ชั่วโมงมีค่าลดลงเป็นเส้นตรง ($P<0.001$) เมื่อเพิ่มระดับของอินนูลินความเข้มข้นของกรดไขมันที่ระเหยได้ เมื่อสิ้นสุดการบ่มที่ 24 และ 48 ชั่วโมงพบว่า ค่าสัดส่วนของอะซิเตทต่อโพรพิโอเนตลดลงอย่างมีนัยสำคัญทางสถิติ ($P<0.001$) เมื่อเปรียบเทียบกับกลุ่มการทดลองที่ไม่ได้เสริมอินนูลินที่ระยะเวลาการหมัก 24 และ 48 ชั่วโมง และเมื่อสิ้นสุดระยะเวลาการบ่มที่ 24 ชั่วโมงพบว่า การเสริมแป้งมีผลต่อค่าอะซิเตท บิวทีเรท และสัดส่วนของอะซิเตทต่อโพรพิโอเนต แต่ที่ระยะเวลาการบ่มที่ 48 ชั่วโมงไม่มีอิทธิพลต่อค่าบิวทีเรท วาเลอเรต และไอโซบิวทีเรท อย่างไรก็ตามในการศึกษานี้พบว่า มีการลดลงของอะซิเตทและการเพิ่มขึ้นของโพรพิโอเนต และบิวทีเรทเมื่อเปรียบเทียบกับกลุ่มที่ไม่เสริมอินนูลิน ปริมาณของแบคทีเรียทั้งหมด *Bifidobacterium* และ *Lactobacillus* เพิ่มขึ้นแบบเป็นเส้นตรงเมื่อเพิ่มระดับของอินนูลิน แต่ *Escherichia coli* และ *Clostridium difficile* ลดลงเป็นแบบเส้นตรงเมื่อระดับของอินนูลินเพิ่มขึ้น

การทดลองที่ 2 การศึกษานี้เป็นผลของอินนูลินจากแก่นตะวันในแพะนม ลูกแพะที่ใช้ในการทดลองเป็นสายพันธุ์ซาแนนจำนวน 20 ตัว และแบ่งกลุ่มการทดลองออกเป็น 5 กลุ่ม ได้แก่ 1) ลูกแพะได้รับนมแพะ 2) ลูกแพะได้รับนมผงทดแทนที่ไม่มีการเสริมอินนูลิน 3) ลูกแพะได้รับนมผงทดแทนที่มีการเสริมอินนูลิน 0.1% 4) ลูกแพะได้รับนมผงทดแทนที่มีการเสริมอินนูลิน 0.2% และ 5) ลูกแพะได้รับนมผงทดแทนที่มีการเสริมอินนูลิน 0.3% แต่ละกลุ่มมีลูกแพะทั้งหมด 4 ตัว และ

วางแผนการทดลองแบบสุ่มสมบูรณ์ (CRD) ผลการทดลองพบว่า คะแนนมูลมีค่าแตกต่างกันในลูกแพะที่อายุ 7 ถึง 56 วัน ($P<0.05$) ในขณะที่ไม่พบความแตกต่างของค่าคะแนนของมูลในลูกแพะที่อายุ 70 ถึง 90 วัน ($P>0.05$) เมื่อลูกแพะอายุ 35-90 วันพบว่า ลูกแพะกลุ่มที่ได้รับนมแพะ 0.2 และ 0.3% อินนูลินในนมผงทดแทน มีน้ำหนักตัวมากกว่ากลุ่มที่ได้รับนมผงทดแทนที่ไม่มีการเสริมอินนูลิน และลูกแพะที่ได้รับนมผงทดแทนที่มีการเสริมอินนูลิน 0.1% ผลของปริมาณเม็ดเลือดขาวของกลุ่มที่มีการเสริมอินนูลินในนมผงทดแทนมีค่าต่ำกว่ามาตรฐาน โดยพบว่า อินนูลินมีผลต่อลักษณะทางสัณฐานวิทยาของลำไส้เล็ก ความยาวของลำไส้เล็ก และลักษณะทางเดินอาหารของลูกแพะ ความยาวของลำไส้เล็กส่วนเจจูนัมและดูโอดีนัมมีแนวโน้มลดลงในลูกแพะกลุ่มที่ไม่มีการเสริมอินนูลินในนมผงและมีค่าเพิ่มขึ้นในกลุ่มที่ได้รับนมแพะและกลุ่มที่มีการเสริมอินนูลินในนมผงทดแทนที่ระดับ 0.1 0.2 และ 0.3% เมื่อเปรียบเทียบกับกลุ่มที่ไม่มีการเสริมอินนูลิน ($P<0.05$) ในการศึกษาพบว่า ปริมาณ *Clostridium* และ *E. coli* ของลูกแพะกลุ่มที่ไม่มีการเสริมอินนูลินในนมผงทดแทนเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ แต่ปริมาณ *Lactobacillus* และ *Bifidobacteria* มีปริมาณลดลงอย่างมีนัยสำคัญทางสถิติ

สาขาวิชาเทคโนโลยีการผลิตสัตว์
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ลายมือชื่อนักศึกษา กนกวรรณ ทำชาร
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ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

KANOKWAN KAMKAJON : UTILIZATION OF PLANT INULIN IN YOUNG RUMINANTS. THESIS ADVISOR : ASSOC. PROF. PRAMOTE PAENGKOUM, Ph.D., 135 PP.

INULIN/YOUNG RUMINANT/DAIRY GOAT/PREBIOTIC/REAL TIME PCR

The purposes of the present study were to investigate the potential of inulin from plants, Chicory (*Cichoriumintybus* L.) and Jerusalem artichoke (*Helianthus tuberosus* L.) for young ruminants.

Experiment I, was conducted on inulin from the chicory level on the abundance of bacteria using fresh fecal samples of Jersey calves incubation *in vitro* culture. Two levels of starch (0 and 1% starch) and 4 levels of inulin from chicory (0, 1, 2 and 4% inulin, respectively) in a 2 x 4 factorial in completely randomized design (CRD). In the culture, sterile medium + fecal slurry + treatment were carried out in triplicate and incubated at 37 °C for 24, 48 hr in a shaking incubator. At the end of 24 hr of fecal fermentation, inulin was increased the total gas production linearly when increasing the percentage of inulin which is simultaneously reflected when increased time of incubation from 0 to 24 hr. In contrast, the pH values were decreased linearly ($P<0.001$) with an increasing percentage of inulin. The volatile fatty acid (VFA) concentrations at the end of 24 and 48 hr of incubation influence the levels of inulin. The ratio of acetate to propionate (A/P ratio) was significantly ($P<0.001$) reduced when compared with 0% inulin at 24 and 48 hr. The addition of starch influenced acetate, butyrate and A/P ratio ($P<0.001$) at 24 hr but did not influence butyrate, valerate and iso-butyrate at 48 hr. Nevertheless, in this study acetate was reduced significant and increased propionate and butyrate proportions when compared with

0% inulin. The abundance of the total bacteria, *Bifidobacterium* and *Lactobacillus*, were increased linearly with increasing levels of inulin but *Escherichia coli* and *Clostridium difficile* were decreased linearly with increasing levels of inulin used quantify specific real-time PCR assays.

Experiment II, this study on the effect of dietary inulin from Jerusalem artichoke on young dairy goats. Twenty newborn Saanen kids were assigned to the five treatments. They are as follows : Goat milk, 0, 0.1, 0.2, and 0.3% inulin was added to milk replacer, respectively and four replicates for a completely randomized design (CRD). There were differences in the fecal scoring on days 7 to 56 ($P<0.05$) whereas, no difference on days 70 to 90 ($P>0.05$) was detected in the groups. Animals fed goat milk, 0.2 and 0.3% inulin was added to the milk replacer, had significantly ($P<0.05$) higher body weight (BW) than 0 and 0.1% inulin while the control treatment led to intermediate values at 35-90 days. Total white blood cell (WBC) on 0% inulin was higher when compared with the standard. To evaluate whether inulin influenced small intestinal morphology, the jejunum and duodenum length showed an overall tendency to be decreased in 0% inulin and to be increased in goat milk, 0.1, 0.2 and 0.3% inulin was added to milk replacer when compared to the 0% inulin ($P<0.05$). In the current study, animals fed 0% inulin had significantly higher CFU of fecal suspension for total clostridium, *E. coli*, *Lactobacillus* and *Bifidobacteria* than other treatments at 30 days.

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Student's Signature kanokwan
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Co-advisor's Signature

ACKNOWLEDGMENTS

I would like to say that I am indebted to many people for making the time working on my Ph.D., an unforgettable experience.

First of all, I would like to express my deepest appreciation to my advisor, Assoc. Prof. Dr. Pramote Paengkoum for providing me the opportunity to study towards my Ph.D. degree in Animal Production Technology, and continuous guidance, unconditional support and encouragement me to complete this thesis.

I am very grateful to my co-advisory committee members, Prof. Dr. Zhongtang Yu, of Department of Animal Sciences, College of Food, Agricultural and Environmental Sciences, The Ohio State University, Columbus, OH, USA. For giving me the opportunity to work for the first time with Real-time PCR and microbiology technique, unconditional support and continuous guidance helped me throughout of the experiment in his laboratory.

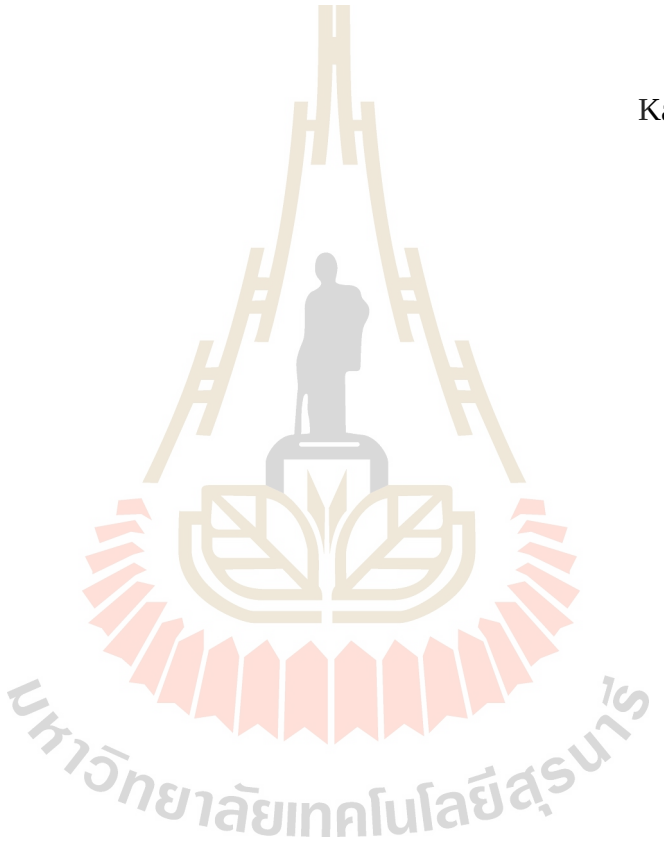
I would like to special thanks to my co-advisory committee members Dr. Siwaporn Paengkoum, Program in Agriculture, Faculty of Science and Technology, Nakhon Ratchasima Rajabhat University, Nakhon Ratchasima, Thailand., for devotion of his time in the co-generation of ideas and earnest work.

I would like to thank graduate student and staff of Department of Animal Sciences, College of Food, Agricultural and Environmental Sciences, The Ohio State University, Columbus, OH, USA, to Lingling Wang, Yueh-Fen Li, Bethany Denton Tansol Park, and my friends in animal production technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand., for helpful suggestion.

Additionally, this research would have never happened if without financial assistance. I very much wish to express my gratitude to The Royal Golden Jubilee (RGJ) Ph.D. Scholarship (Grant No. PHD/0110/2552) for my study.

Finally, I most gratefully my parents who are always on my mind, love and care throughout my life, understanding, and dedicated cooperation, none of this would have been possible.

Kanokwan Kamkajon



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LIST OF ABBREVIATIONS

ADF	=	Acid Detergent Fiber
BW	=	Body Weight
CP	=	Crude Protein
DCL	=	Duodenum Crypt Length
DM	=	Dry Matter
DVL	=	Duodenum Villuos Length
EE	=	Ether extract
FOS	=	Fructo-oligosaccharide
ICL	=	Ileum Crypt length
IVL	=	Ileum Villuos length
JCL	=	Jejunum Crypt length
JVL	=	Jejunum Villuos length
MOS	=	Mannan-oligosaccharides
NDF	=	Neutral Detergent Fiber
OM	=	Organic Matter
PBS	=	Phosphate Buffered Saline
RBC	=	Red Blood Cell
VFA	=	Volatile fatty acid
WBC	=	White Blood Cell

CHAPTER I

INTRODUCTION

Diarrhea in young (neonatal) lambs and kids, despite improvements in management practices and prevention and treatment strategies, diarrhea is still the most common and costly disease affecting neonatal small ruminants. Neonatal calf diarrhea is an important cause of morbidity and mortality in young ruminants (Gaggia et al., 2010). In a recent survey, it was reported that 7.8% of dairy heifers died prior to weaning and more than half died as a result of scours or bacterial enteritis (USDA, 2007). A study at the U.S. Sheep Experiment Station (Dubois, ID) showed that diarrhea accounted for 46 percent of lamb mortality. Diarrhea in lambs and goats is a complex, multi-factorial disease involving the animal, the environment, nutrition, and infectious agents. The four major causes of diarrhea in lambs and kids during the first month of life are *Escherichia coli* , rotavirus, *Cryposporidum* sp. and *Salmonella* sp. *E. coli* scours are most common (Susan Schoenian., 2007). Diarrhea is common in newborn calves, lambs and kids. The clinical presentation can range from mild diarrhea without systemic disease to profuse, acute diarrhea associated with rapid dehydration, severe disturbance of acid-base and electrolyte balance, and death, sometimes in as few as 12 hr. (Walter Gruenberg et al., 2014). Diarrhea is a common complaint in calves and other young ruminants, particularly in the first few months of life. Many of the pathogens and management practices that cause diarrhea in calves also affect lambs, goats and modified ruminants such as llamas (Lisa Williamson, 2002). Farm animals are often subjected to physiological, psychological, and

environmental stressors leading to dysfunction of the intestinal barrier and an increase in intestinal permeability resulting in an imbalance in the intestinal ecosystem, including microbial composition and susceptibility to enteric pathogens (Gaggia et al., 2010; Gareau et al., 2009). Beneficial bacteria, such as *Lactobaccilli* and *Bifidobacteria*, have been shown to decrease when stressing factors occur (Si et al., 2004). Since the most studied external factor that influences establishment of the intestinal microbiota is diet (Mackie et al., 1999), methods to increase the concentrations of these beneficial bacteria through diet could give producers an alternative to antimicrobial use.

For several decades, the use of sub-therapeutic levels of antibiotics in animal feeds has been a common practice in many countries in order to improve growth performance and prevent from the adverse effects of pathogenic and non-pathogenic enteric microorganisms. However, there are increasing concerns for the public health about the consequences from the use of antibiotics in livestock (Phillips I., 1999). The risk of developing cross-resistance and multiple-antibiotic resistance in pathogenic bacteria both in human and farm animals, has been strongly linked to the therapeutic, metaphylactic or prophylactic uses of antibiotics in human and veterinary medicine, as well as growth promoters in animal feed (Gibson GR and Roberfroid MB., 1995).

There is increasing public and scientific concern about the use of antibiotics as feed additives in animal production. This concern is fueled by the emergence of antibiotic resistance in many human pathogenic bacteria (Manero et al., 2006; Parveen et al., 2006), the release of contaminating residues into the environment (water, soil, etc.) (Yang and Carlson, 2004) and the risk that growth-promoting antibiotic residues may occur in foods of animal origin. For all these reasons, the

European Union (EU) decided that antibiotics used in livestock as production enhancers would be banned from 1 January 2006 (EU regulation no.1831/2003 of the European Parliament and of the Council of 22 September 2003). This ban has led to animal performance problems and a rise in the incidence of certain diseases (Wierup M., 2001; Dibner JJ. and Richards JD., 2005). Thus, there is an urgent need to develop alternatives to antibiotics, especially in EU. As a consequence of the public health concerns and the demand of the farmers to prevent the economic losses, non-antibiotic additives have been developed for prophylactic use against pathogens or as growth promoters. Consequently, there is nowadays a real demand among animal producers for alternative feed additives, and among consumers for more natural and safe products in the human food supply chain. Many substances, such as probiotics, prebiotics, some organic acids involved in metabolic pathways, herbs and plant extracts, can offer some of the benefits that antibiotics provide. Here we will review current feeding and nutritional problems raised by modern ruminant production, and the availability of 'natural' substances and their potential efficacy in solving these problems. Possible limits to the use of these agents will also be discussed (J.-P. Jouany and D. P. Morgavi., 2007).

Inulin is a natural β-(2-1)-linked fructo-oligosaccharide with up to 60 units common in plants used in the Western diet (Van Loo et al., 1995). It has been show to lead to a shift in the intestinal bacteria flora toward more beneficial *Bifidobacterium* (Gibson et al., 1995), to stimulate the immune system (Schley and Field, 2002). In addition, it was shown to enhance growth performance of livestock (Van Loo, 2007).

The digestive system of the young goat is very similar to that of the pig and human (explaining the term 'preruminant'). During these first stages of milk feeding,

the abomasum (true stomach) and small intestine play a relatively important role with respect to digestion and nutrition. In young goats, and other young ruminants, the suckling reflex triggers the esophageal groove to close so that milk bypasses the rumen and flows directly in to the abomasum where clotting and some digestion occurs. Milk protein is rapidly digested in the small intestine, as is lactose. If the esophageal groove does not close, for whatever reason, then milk goes into the rumen where it 'ferments', allowing digestive upsets to become problems (Anita O'Brien., 1998).

A lot of studies have been conducted to investigate the effects of prebiotics on intestinal microbial populations, digestive and systemic health, immune function and growth performance in non-ruminant. Therefore in ruminant animals (cattle, buffalo, goat, sheep, deer), some of microorganisms in the rumen can digest inulin, thereafter the efficiency of inulin in digestive system decreases. However, there is no information on the effects of supplemental inulin and other prebiotic compounds in the ruminant. The objectives of this study are to investigate the effects of feeding inulin in milk replacer on fecal score, fecal pH, BW, haematological traits, selected health parameters and the incidence of diarrhea.

1.1 Research objectives

1.1.1 To study the effect of inulin from chicory level on the abundance of bacteria and their fermentation using *in vitro* of calve fecal samples

1.1.2 To study the effect of inulin from Jerusalem artichoke on the health status of young dairy goats.

1.1.3 To study the effect of inulin from Jerusalem artichoke on intestinal histological of dairy goats.

1.2 Research hypothesis

1.2.1 The abundance of *lactobacillus* and *bifidobacterium* will be increased by increased the level of inulin.

1.2.2 The health status will be increased by inulin from Jerusalem artichoke supplementation of dairy goats.

1.2.3 The histological in the intestine will be increased by inulin from Jerusalem artichoke by such supplementation of dairy goats.

1.3 Scope and limitation of this study

The goat's milk is beneficial to human's health similar to breast milk and is more expensive than dairy cow's milk. In addition, it can replace dairy milk for babies who are allergic to dairy milk. Currently, the dairy goat's farmers face with very serious problems. In dairy goat farming, the young goats often show sign of diarrhea due to less immunity. These problems are of critical importance to dairy goats farming because such problems will affect the production. These researches should help to reduce such problems occurring with dairy goats as well.

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CHAPTER II

LITERATURE REVIEW

2.1 Dairy goat farming in Thailand

Goat raising has long been associated with Thai Muslim communities. The distribution of goat population is associated with the areas where Thai Muslims live; the southernmost region and outskirts of Bangkok. The dairy goat sector is relatively new and small compared to other livestock species meat goat. The distribution of goats is rather related to demographic than climate conditions. Dairy goat number is currently estimated below 5% of the total number of goats which is approximately five hundred thousand heads. Importation of exotic dairy goat breed of Saanen was in 1948 and other dairy goat breeds were imported by private companies and government agencies. Saanen is the most popular among dairy goats. Other dairy breeds found in small numbers are Toggenburg, Alpine and Anglo-Nubian and their crossbreds with Thai native goats (Sansak N., 2016). However, goat milk was not popular during the time of first introduction. Later, the Department of Livestock Development (DLD) imported Saanen goats from Australia and Netherlands to the country in 1984 - 1985 and other dairy goat breeds were imported by private companies.

The number and popularity of dairy goat have been slightly increased in last the ten years. According to the livestock population survey by the center of information technology (DLD), Fifty-four percent of the total 440,277 goats were in

the Southern part of Thailand, most of which were Thai native goats. However, Central Thailand recorded a rapid increase over the last decade, primarily as a result of the development of commercial goat farms to cater for the demand in around Bangkok areas. The latest statistic shows that about 36% of goats in Thailand are found in the central with the remaining 10% located in the Northern and Northeastern regions. Dairy goat production has been quite small and stable with a possibility to grow slowly (Sansak N. and Suwit A., 2014).

The overall average number of dairy goat farm size in the Thailand is around 20 - 30 goats per farm (Sansak N., 2016). The sizes of dairy goat farm are different in different regions being the largest in the central area. According to 2011 survey, the farm sizes of dairy goats in the central part of Thailand are larger than the rest of the country. Dairy goat farms in Central Thailand averaged 43 heads/farm compared the North (24 heads/farm), Northeast (14 heads/farm) and Southern Thailand (ranging from 5 to 7 goats/farm). Larger farm size indicates the operation of the farm as well that is a larger farm size tends to adopt a more intensive farming system (Sansak N. and Suwit A., 2014).

The majority of the goat milk producers have other main occupations in crops such as rubber or oil palm plantations and fruit orchards. Annual goat milk production in Thailand is very low ranging from two to five thousand tons per year compared to a million tons of cow milk. The price of raw goat milk in 2015 was around 50 - 60 Baht (2 US$) per kg which was approximately three times higher.

2.2 Antibiotic in the animal

In an effort to maximize health and performance of neonatal dairy calves, antimicrobials have been incorporated into the milk replacer for more than 50 years. Antimicrobials are defined as any substance that kills or inhibits the growth of microorganisms. Antibiotics are substances produced by microorganisms that kill or inhibit the growth of other microorganisms (USDA, 2010). These two terms are often used interchangeably.

Since the 1940's antibiotic growth promoters are an integral part of livestock production. Although the precise mechanisms of growth promotion triggered by antimicrobial agents still remain unclear, their use as a supplement in feed has been a defining characteristic of modern livestock farming. Growing concerns about antibiotic resistant bacteria and their transfer from animals to humans has led to the ban of antibiotic growth promoters and the subsequent quest for alternatives. (Pancosma. 2016) However, with the inclusion of antimicrobials, there is a concern of bacterial resistance to these compounds.

Nowadays, there is an increasing concern for the public health about the consequences from the long and increased use of antibiotics in livestock production. The use of antibiotics in animal feed as growth promoters has been complete banned by the European Union (EU) since 2006 (EC Regulation No. 1831/20031), based on their possible negative consequences for animal health and food safety (EFSA, 2009; Fernando U.et al., 2007), based on their possible negative effects for human and animal health. Growth promoters' removal has led to animal performance problems and a rise in the incidence of certain animal diseases. Thus, there is an urgent need to find alternatives to antibiotics, especially in EU. (Papatsiros VG., et al 2012). This

ban has led to animal performance problems and a rise in the incidence of certain diseases (Wierup M. 2001; Dibner JJ and Richards JD., 2005). Thus, there is an urgent need to develop alternatives to antibiotics, especially in EU. As a consequence of the public health concerns and the demand of the farmers to prevent the economic losses, non-antibiotic additives have been developed for prophylactic use against pathogens or as growth promoters.

Due to the modern consumers' concern about the potential development of antibiotic-resistant bacteria as well as at the same time the need to prevent economic losses of the farmers, alternatives to antibiotics has been developed to prevent the health problems and to improve the growth performance in farm animals. Due to the full ban of antibiotic use in EU there is an urgent need to find alternatives to in-feed antibiotics. New strategies and commercial products must be developed to improve animal health and performance, based on their safety, efficacy and cost effectiveness (Papatsiros VG., et al 2012).

In June of 2010 the FDA published a draft guidance informing the public of their concerns on the routine use of medically important antimicrobial drugs in feed of food-producing animals. The report stated that "antimicrobial resistance, and the resulting failure of antimicrobial therapies in humans, is a mounting public health problem of global significance." The FDA finds the use of medically important antimicrobial drugs in food producing animals for use as growth promotants and improved feed efficiency an injudicious use. However uses associated with the treatment, prevention, or control of specific diseases including incorporation in feed and water are considered to be necessary uses for assuring the health of food producing animals (US-DHHS, 2010). This being said some drugs have been banned,

others limited in uses, and stricter guidelines are being put in place for any new drugs approved for market. Those already on the market are being reevaluated to determine their effect on antimicrobial resistance in humans.

2.2.1 Antibiotic in the young animals

The use of medicated milk replacers have become a common practice of dairy producers (Visek, 1978). Antimicrobials are used in feedstuffs of young animals for several reasons. At subtherapeutic levels antimicrobials have been shown to prevent, control, and treat enteric infections; while improving growth and overall feed efficiency at a critical time in the animal's life. In 2006 it was reported that 49.9 % of all heifers received medicated milk replacers at some point prior to weaning (USDA, 2007).

Donovan et al. (2002) reported that the addition of antibiotics to the milk replacer, starter, or water of young calves improves feed efficiency, growth, starter consumption, and phagocytic efficiency. Morrill et al. (1977) reported that the inclusion of antibiotics in the milk of calves resulted in increased gain as well as feed consumption even when there was no presence of digestive disease. Berge et al. (2005) reported that calves fed a medicated milk replacer had decreased overall morbidity and increased weight gain compared with calves fed a non-medicated milk replacer. Losinger et al. (1995) reported a reduced shedding of entero-pathogens, while both Quigley et al. (1997, 2002) reported a reduction in the severity and length of scouring, improved body weight gains, and improved overall efficiency of calves fed milk replacer supplemented with antibiotics.

In 2006, chlortetracycline and oxytetracycline in combination with neomycin, decoquinate, and lasalocid were the only approved antimicrobial agents that could be

used in medicated milk replacers (USDA, 2007). It was reported that 49.5% of operations used oxytetracycline in combination with neomycin in medicated milk replacers, and oxytetracycline and decoquinate were fed on nearly one of five operations (USDA, 2010).

2.2.2 Development of Antimicrobial Resistance

Dibner and Richards (2005) noted that pathogens, such as *Camplyobacter* and *Salmonella* can be transmitted along the food chain and can be a source of human illness. Feeding even subtherapeutic levels of antibiotics over a long period of time could result in the intestinal bacteria becoming resistant to the antibiotics and when slaughtered, these resistant bacteria can enter the human food chain and cause detrimental illnesses by pathogens that are resistant to normal antibiotic therapies. It was first documented in 1963 when increased levels of resistance were observed in a particular strain of Salmonella typhimurium in several British feedlots. Over a three year period several resistant isolates were subsequently identified (Dewey et al., 1997). Kaneene et al. (2008) reported that feeding non-medicated milk replacer had an increase in antimicrobial susceptibility in *E. coli* and Salmonella, while feeding medicated milk replacer was associated with decreased susceptibility of *E. coli* and Salmonella from fecal and calf pen samples. Langford et al. (2003) found that resistance of gut bacteria to antibiotics increased with increasing concentrations of penicillin in milk fed to dairy calves. However, Berge et al. (2005) concluded that removal of antibiotics from milk replacers may have a significant negative impact on calf health in the absence of adequate passive transfer of immunity.

2.2.3 Antimicrobial Resistance

Antimicrobial efficacy or the lack of efficacy of a drug for treatment of a disease agent for which the drug was previously effective was recognized soon after the widespread use of antibiotics began (Kaneene et al., 2008). One study estimated that the direct hospital cost of managing antibiotic resistance in the United States is $100 million to $10 billion per year, and the Office of Technology Assessment estimated in 1992 that the minimal hospital cost of 5 types of nosocomial infection due to antibiotic resistance were $4.5 billion per year (US Office Tech, 1995). Since then several health and medical organizations have been investigating the possibility that the subtherapeutic use of antimicrobials could be increasing the level of antibiotic resistance in not only animals but humans as well. Reports by Fey et al. (2000) and Tollefson et al. (1999) suggest that continued use of antimicrobials in animal agriculture may contribute to increased risk of antibiotic-resistant bacteria of medical importance. Several products have been tested to determine efficiency of reducing bacterial enteritis and improving feed efficiency while recognizing that a properly functioning intestinal tract can greatly reduce the prevalence of enteric infections in young calves.

2.3 General information of Jerusalem artichoke

Jerusalem artichoke (*Helianthus tuberosus* L.) is familiar to many as a weed, but has some potential as a crop plant. Native to the central regions of North America, the plant can be grown successfully throughout the U.S. under a variety of temperature and rainfall regimes. Several North American Indian tribes used Jerusalem artichoke as food prior to the arrival of European settlers. The explorer

Champlain took Jerusalem artichokes from North America to France in 1605. By the mid-1600s it was widely used as a human food and livestock feed there (D.R. Cosgrove et al., 2016).

(a) (b)

Figure 2.1 (a).Jerusalem artichoke flower (b).Jerusalem artichoke tuber

Source : (a) http://www.rainamthip.com/default.asp?content=spagedetail&cid=9512

(b) http://bookmuey.com/?page=Jerusalem_Artichoke.html&admintool=no

Jerusalem artichoke is a herbaceous plant, eaten at its bulb, the bulb of Jerusalem artichoke has the important ingredients, inulin, fructo-oloigosaccharide (FOS), protein, fiber, calcium and phosphorus. Jerusalem artichoke can be adapted to growing condition in Thailand and tend to increase planting both for human diet and also by-products or co-products for animals. Its tuber contains high amount of dietary fiber namely inulin and FOS (15.28 and 5.96 g/100 g fresh weight, respectively) (Tanjor et al. 2012). Jerusalem artichoke contains 15-20% of inulin and FOS, and it is considered to be prebiotics which have been proposed to improve health by stimulation of beneficial bacteria in the intestine of humans and animals. Inulin is carbohydrate in the form of fructan. It is fiber source which is not digested in

digestive system in (stomach and intestine) single stomach animal or human. Therefore inulin will fall into colon and it has benefit for body growth. It increases the number of beneficial microorganisms to health, such as *Lactobacillus* and *Bifidobacteria* (Younes et al., 1995; Kaur and Gupta, 2002).

2.4 General information of inulin

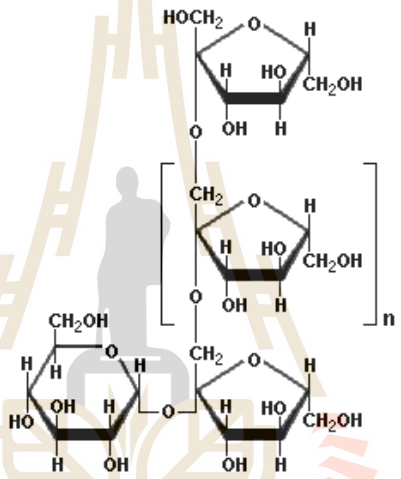

Figure 2.2 Chemical structure of inulin compounds.

Source : http://www.scientificpsychic.com/fitness/carbohydrates1.html

It was first discovered as a “peculiar substance” by German scientist Rose in the year 1804 from the hot water extract of roots of plants (Inulahelenium) belonged to Compositae family. Later on, the term “inulin” was coined by Thomson in 1818. Chemically it is fructan made up of fructose units linked by β- 2, 1 linkage (Fig. 2.2) of which terminal sugar is obviously glucose. Inulin is represented by general formula GF_n, while oligofructose (OF) by F_m and/or GF_n; ‘G’ is glucosyl unit, ‘F’ is fructosyl unit, ‘n’ is the number of fructose units linked to terminal glucose units and ‘m’ is the

number of fructose units linked to each other in the carbohydrate chain (Franck, 2000; Niness, 1999). In case of inulin, 'n' is greater than 2 and less than 60. OF, obtained by partial hydrolysis of inulin can be a mixture of both GF_n and F_m molecules with DP varying from 2 to 7 or 8 to10 depending on the products' brand (Franck, 2000). Nonetheless only GF_ntype OF could be produced from inulin by enzymatic hydrolysis or by trans-fructosylation of sucrose molecule in the presence of fructosyltransferase (Sangeetha et al., 2005). In chicory inulin, the number of fructose units linked to a terminal glucose can vary from 2 to 70 units. By means of an endo-inulinase inulin is hydrolysed into a DP between 2 and 8 (average DP=4) called OF (D. Bosscher, et al 2009). First elongation of sucrose with one fructose molecule leads to generation of fructooligosaccharides termed as 1-kestose and further elongation leads to production of 1-nystose and so on (Samanta et al., 2010). The degree of polymerization (DP) refers to the number of repeating units (fructose) present in the prebiotic inulin or OF and depends upon plant source, growing stages, climatic conditions, post-harvest processing etc. Inulin may also contain minor amounts of $F_m$fructans (F2), although GF_nfructans with DP from 2 to 60 are predominant.

Inulin is a polydisperse non-starch polysaccharide naturally occurring as a storage carbohydrate in some 36,000 plant species (Patkai and Barta, 2002) and the list include roots of chicory (15-20%), burdock (3.5 -4%), salsify (4-11%), yacon (3-19%), murnong (8-13%), bulbs of onion (2-6%), garlic (9-16%), leek (3-10%), camas (12-22%), tubers of Jerusalem artichoke (16-20%), leaves of dandelion (12-15%), artichoke (3-10%) in addition to a number of cereals and fruits. Presently most of the inulin is produced from roots of chicory in which chain length ranges from 2 to 60, with an average degree of polymerization 10. OF is made by 2 to 8 molecules of

fructose units, terminally linked with a glucose moiety. The presence of β-2,1-osidic bonds either inulin or OF make the prebiotic indigestible by mammalian enzymes (Gibson et al., 2004).

Silva (1996) reported that inulin is water soluble, the solubility being temperature dependent. At 10°C its solubility is about 6% whereas at 90°C it is about 35%. Jerusalem artichoke tubers with 14-19% inulin can be a valuable source of inulin (Vanloo, et al., 1995). Due to their β (2,1)-bonds, inulin is resistant to enzymatic hydrolysis in the upper gastrointestinal tract of humans and monogastric animals. When undigested inulin reaches the colon it is fermented by colonic microflora, which causes selectively stimulating the growth of certain groups of bacteria. Therefore, inulin is considered the archetypal prebiotic (Fischbein, 1988; Niness, 1999; Roberfroid, 1998; Wang and Gibson, 1993; Hakan ozturk, 2008). Inulin is used successfully in monogastric animals, but not in ruminants. The process of fermentation that occurs in the colon of monogastric animals is essentially identical to that occurs in the forestomachs of ruminants. At present, few studies have investigated the direct effects of inulin on rumen metabolism (Hakan ozturk, 2008).

Inulin type fructans, are the best documented oligosaccharides for their effect on intestinal *Bifidobacteria* and are considered important prebiotic substrates (Vos et al., 2006). Inulin is not only important because of their low calorie properties, but also because of the ‘‘bifid’’ factor, which implies in the regulation of the intestinal flora. Recently, inulin was identified as an ingredient for fat or sugar substitution. Due to these properties, food and pharmaceutical industries have found applications for inulin. It acts in the organism in a similar way as dietary fibers, contributing to the improvement of the gastrointestinal system conditions (Roberfroid et al., 1993).

Marchetti (1993); Gaafar et al. (2010) reported that, inulin is a natural polymer that not hydrolysable by the intestinal enzymes, because it has β (2-1) link which is not be hydrolyzed. So it could be considered a calorie free fiber, although some calories may occur due to the digestible fermentation of these by products in the colon. FOS and inulin are composed of short chains of fructose molecules (Roberfroid, 1993). Administration of FOS or inulin can serve as food (Cieoelik et al., 2002) for and increase the number of *bifidobacteria*, *lactobacilli* and certain butyrate-producing bacteria (Hold et al., 2003) in the colon while simultaneously reducing the population of harmful bacteria such as the *Clostridium* perfringens group (Gibson et al., 1995). Other benefits noted with FOS or inulin supplementation include increased production of beneficial short-chain fatty acids such as butyrate, increased absorption of calcium and magnesium, and improved elimination of toxic compounds (Tomomatsu, 1994; van den Heuvel et al., 1999; Yildiz et al., 2006).

Inulin, OF and FOS are fully metabolized by the colonic microflora. The end products of fermentation are gases (such as carbon dioxide and hydrogen), lactate, and short-chain fatty acids (including acetate, propionate, and butyrate). Increased hydrogen concentrations can be observed by breath hydrogen testing. Colonic bacterial fermentation of inulin-type prebiotics, and the by-products produced, acidify the colonic content, increase bacterial biomass (and consequently fecal mass), and modify the composition of the microflora. The primary stimulating effect of prebiotics on gut ecology is stimulation of *Bifidobacteria* species growth. Although inulin, OF, and FOS are bifidogenic, the effects on other gut microorganisms as well as pathogenic organisms are inconsistent (Greg Kelly, 2008).

Table 2.1 Inulin content (% of fresh weight) of plants that are commonly used in human nutrition.

Source	Edible parts	Dry solids content	Inulin content
Onion	Bulb	6-12	2.0-6.0
Jerusalem artichoke	Tuber	19-25	14.0-19.0
Chicory	Root	20-25	15.0-20.0
Leek	Bulb	15-20*	3.0-10.0
Garlic	Bulb	40-45*	9.0-16.0
Artichoke	Leaves-heart	14-16	3.0-10.0
Banana	Fruit	24-26	0.3-0.7
Rye	Cereal	88-90	0.5-1.0*
Barley	Cereal	NA	0.5-1.5*
Dandelion	Leaves	50-55*	12.0-15.0
Burdock	Root	21-25	3.5-4.0
Camas	Bulb	31-50	12.0-22.0
Murnong	Root	25-28	8.0-13.0
Yacon	Root	13-31	3.0-19.0
Salsify	Root	20-22	4.0-11.0

NA, data not available. *Estimated value. (Van Loo et al., 1995)

2.5 Overview of inulin in animal

At birth, the digestive system of the young goat is very similar to that of the pig and human (explaining the term 'preruminant'). During these first stages of milk feeding, the abomasum (true stomach) and small intestine play a relatively important

role with respect to digestion and nutrition. In young goats, and other young ruminants, the suckling reflex triggers the oesophageal groove to close so that milk bypasses the rumen and flows directly in to the abomasum where clotting and some digestion occurs. Milk protein is rapidly digested in the small intestine, as is lactose. If the esophageal groove does not close, for whatever reason, then milk goes into the rumen where it 'ferments', allowing digestive upsets to become problems (Anita O'Brien., 1998).

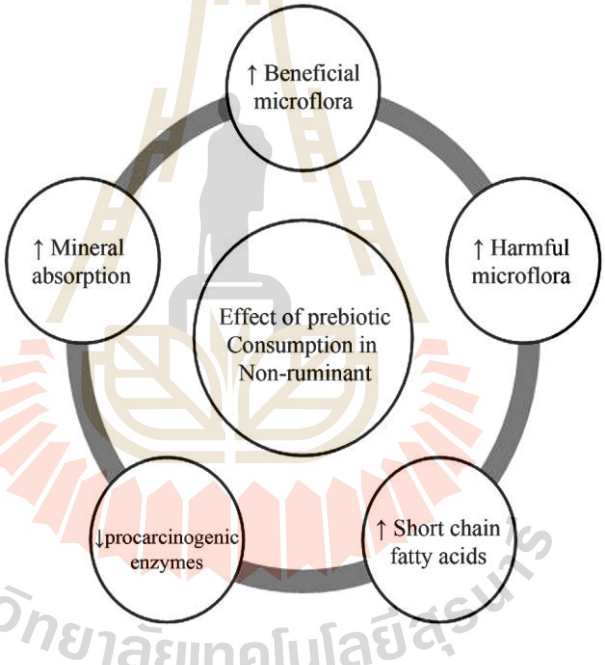

Figure 2.3 Schematic diagram on effect of prebiotic consumption in non-ruminants.

Source : Samanta et al., 2013

Samanta et al. (2013) reported the foregut and hindgut houses millions of diverse groups of microflora namely bacteria, fungi, yeasts, phage particles, archaeaetc with the exception that protozoa are supposed to be present only at foregut and absent at hindgut. Prebiotics are fermented by number of rumen bacteria for its

utilization as source of energy (Cota and Whitefield., 1998; Samanta et al., 2012). The encouraging results of prebiotics on human health especially the gut microbial ecology have prompted ruminant researchers to explore its potentiality on different livestock species like cattle, buffalo, sheep etc. Ruminants are exposed to different kind of stress on different occasions like weaning, transportation, which adversely affect the health of the livestock, resulting in diarrhea, off fed, depression of growth, impaired intestinal morphology etc (Fraser et al., 1998; Nabuurs, 1998). Under such situations, ecological treatment through prebiotic may be potential alternatives to overcome the gut associated problems of livestock. Presently frugal information is available on the effects of prebiotic in ruminant animals. All the rumen hemicellulolytic bacteria are capable to utilize xylooligosaccharides as growth substrate (Cota and Whitefield, 1998). These are *Butyrovibrio fibrisolvens*, *Eubacterium ruminantium*, *Ruminococcus albus* etc. The rumen pH remained unchanged (6.7), when prebiotic is given to Holstein cows maintained on orchard grass silage or alfalfa silage (Santoso et al., 2003). In ruminant species, the above pH (6.6 to 6.8) is ideal for growth and multiplication of useful plant biomolecules degrading bacteria (Samanta et al., 2003). Some of the researchers did not notice any significant changes of rumen pH in steers supplemented with prebiotics; however, they recorded significantly higher oxidation reduction potential (Mwenya et al., 2004). The rumen ammonia nitrogen concentration was slightly lower in prebiotics supplemented Holstein cows and steers, which might be due to the utilization of ammonia for microbial protein synthesis in the rumen (Mwenya et al., 2005; Santoso et al., 2003). Lower rumen ammonia nitrogen concentration in sheep was also observed as a result of prebiotic administration, which may be due to the suppression

of ammonia producing bacteria (Mwenya et al., 2004). Inclusion of inulin in the milk replacer of pre-ruminant calves leads to significantly higher live weight gains, better feces consistency (Kaufhold et al., 2000; Verdonk and Van Leeuwen, 2004). It is postulated that increase in body weight might be ascribed due to increased fermentation at the small intestine followed by increased flow of microbial nitrogen at large intestine, stable microflora composition at rumen, small and large intestine of calves (Verdonk et al., 1998). The fermentation of inulin is faster at pH 6.0 than at neutral pH by rumen inoculums obtained from sheep maintained on sole forage diets (Flickinger et al., 2003). The diet of calves supplemented with OF resulted in decreased population of fecal *E. coli* and total anaerobic microflora while *Bifidobacteria* population exhibited increasing trends (Bunce et al., 1995a). This might be attributed by beneficial effects brought out through the consumption of prebiotics followed by their fermentation at hindgut of calves. Incorporation of OF in the milk replacer of calves resulted in improved body weight gains, feed conversion efficiency with reduction in the incidence of diarrhea and firmer feces (Mul, 1997). Incorporation of FOS at a concentration of 0.5% to 1% of total mixed ration (w/w) significantly improved the organic matter and dry matter digestibility of total mixed ration by virtue of modulation of rumen metabolic profile (Samanta et al., 2012).

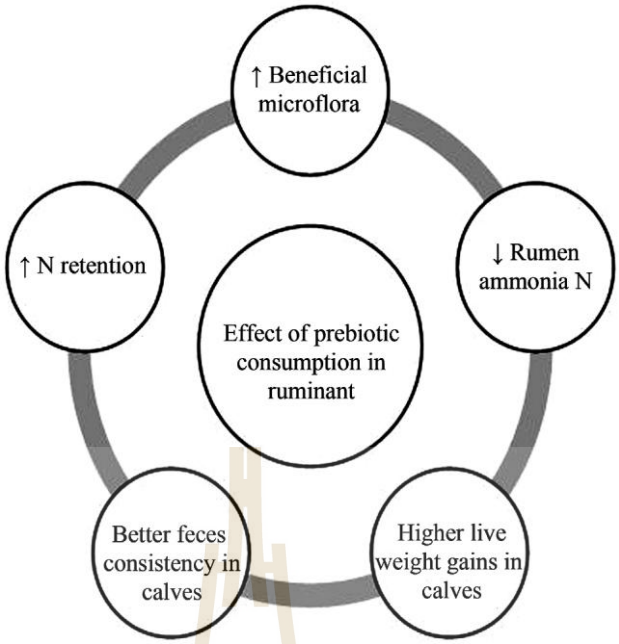

Figure 2.4 Schematic diagram on effect of prebiotic consumption in ruminant.

Source : Samantaet al., 2013

2.6 Probiotic in the ruminant

The concept of probiotics goes back more than 100 years to Elie Metschnikoff who proposed that bacteria in fermented milk products may be able to control bacterial fermentation in the intestinal tract of men and are thus health promoting (E. Metchnikoff, 1908). Probiotics are dietary supplements containing bacteria or yeast, and the use of these is based on the concept that feeding “beneficial” microbes to livestock subjected to stress conditions may prevent establishment of undesirable microorganisms while helping to re-establish normal gut microflora, thus benefiting animal health and performance (Gaggia et al., 2010). Probiotics act mainly via modifications of intestinal bacterial populations and their effectiveness depends on the microbial status of a group of animals and the individual animal. Probiotics are generally recommended in ruminants nutrition whenever a risk of rumen function

exists, in order to improve anaerobiosis, stabilise pH and supply nutrients to microbes in their microenvironment. Probiotics are recommended in young ruminants (Ellinger DK et al., 1978; Bruce BB et al., 1979) to prevent diarrhea caused by enterotoxigenic bacteria in the gut and also during weaning period to enhance the rate at which rumen flora and fauna become established. *Lactobacillus acidophilus* alone or in combination with other lactobacilli has been shown to reduce scouring and increase growth rate of calves in some trials (Bechman TJ. et al., 1977; Beeman K., 1985)

Diarrhea in young (neonatal) ruminants

Diarrhea is defined as an increased frequency, fluidity, or volume of fecal excretion. The feces may contain blood or mucous and be smelly. The color of the feces may be abnormal. However, it is not possible to definitively determine the infectious organism by looking at the color, consistency, or odor of the feces. A definitive identification requires a sample for microbiological analysis.

Despite improvements in management practices and prevention and treatment strategies, diarrhea is still the most common and costly disease affecting neonatal small ruminants. A study at the U.S. Sheep Experiment Station (Dubois, ID) showed that diarrhea accounted for 46 percent of lamb mortality. Diarrhea in lambs and goats is a complex, multi-factorial disease involving the animal, the environment, nutrition, and infectious agents. The four major causes of diarrhea in lambs and kids during the first month of life are *E. coli*, rotavirus, *Cryposporidum* sp. and *Salmonella* sp. *E. coli* scours are most common. (Susan Schoenian., 2007).

E. coli

E coli is the most important bacterial cause of diarrhea in calves during the first week of life; at least two distinct types of diarrheal disease are produced by

different strains of this organism. One type is associated with enterotoxigenic *E coli*, which has two virulence factors associated with production of diarrhea. Fimbrial antigens enable them to attach to and colonize the villi of the small intestine of neonatal calves in the first days of life. Strains in calves most commonly possess K99 (F5) or F41 fimbrial antigens, or both. These antigens are the focus of immunologic protection. Enterotoxigenic *E. coli* also elaborate a thermostable, nonantigenic enterotoxin (Sta) that influences intestinal ion and fluid secretion to produce a non inflammatory secretory diarrhea. Diarrhea in calves and lambs also has been associated with enteropathogenic *E coli* that adhere to the intestine to produce so-called attaching and effacing lesions, with dissolution of the brush border and loss of microvillous structure at the site of attachment, a decrease in enzyme activity, and changes in ion transport in the intestine. These enteropathogens are also called "attaching and effacing *E coli*." Some produce verotoxin, which may be associated with a more severe hemorrhagic diarrhea. The infection most frequently is in the cecum and colon, but the distal small intestine can also be affected. The damage in severe infections can result in edema and mucosal erosions and ulceration, leading to hemorrhage into the intestinal lumen. (Walter Gruenberg et al., 2014). *E. coli* scours is an opportunistic disease associated with sloppy environmental conditions and poor sanitation. It is seen in lambs and kids less than 10 days of age, but is most common at 1 to 4 days of age. It usually presents itself as an outbreak in lambs and kids between 12 and 48 hours of age. It is also called watery mouth, because affected lambs salivate and have a cold mouth. Fluid therapy is the mainstay of therapy.

Antibiotics are used for both treatment and prevention of *E. coli* scours in lambs. Spectinomycin oral pig scours medicine is commonly used, though it is not

approved for sheep and goats. Ewes and does can be vaccinated with bovine *E. coli* vaccine before they give birth to increase passive immunity. The use of neomycin in lambs that appear normal may stop the progression of the outbreak. Adequate ingestion of colostrum by newborns decreases the incidence of the disease (Susan Schoenian., 2007).

Abe et al. (1995) reported the addition of probiotics to the diets of preweaned calves increased body weights and decreased incidence of scours. Agarwal et al. (2002) found a reduction in the incidence of diarrhea in calves fed milk fermented with either lactic acid bacteria, or *L. acidophilus* 15 or *S. cervisae* NCDC49. Adams et al. (2008) found an improvement in weight gain and rumen development in young calves supplemented with bacterial and yeast strains, in contrast to Jenny et al. (1991) who reported no significant effects on growth and health of neonatal calves when probiotics were included in the milk replacer. However, a shortcoming of probiotics is that only a small proportion of ingested organisms reach the colon intact due to passage through the stomach and small intestines (Topping et al., 2003).

Figure 2.5 Enterotoxigenic Escherichia coli adhering to the intestine of a 2-day-old calf

Source : J. J. Hadad and Carlton Gyles

C. perfringins types A, B, C, and D can all cause diarrhea in lambs and kids, though type D is the most common agent. With type D, the onset of neurologic signs followed by sudden death is more common in sheep, whereas goats are more likely to show signs of diarrhea before death. Treatment is rarely effective but consists of aggressive supportive care and administration of the antitoxin.

*C. perfringens*type C tends to affect very young lambs (<2 weeks of age) and presents itself as bloody diarrhea, hemorrhagic enteritis, and bloody scours. *Clostridial* diseases are easily prevented in the young by vaccinating pregnant dams about three weeks prior to delivery and subsequent vaccination of offspring. Consumption of adequate, high quality colostrum is important.

2.7 Overview of prebiotic

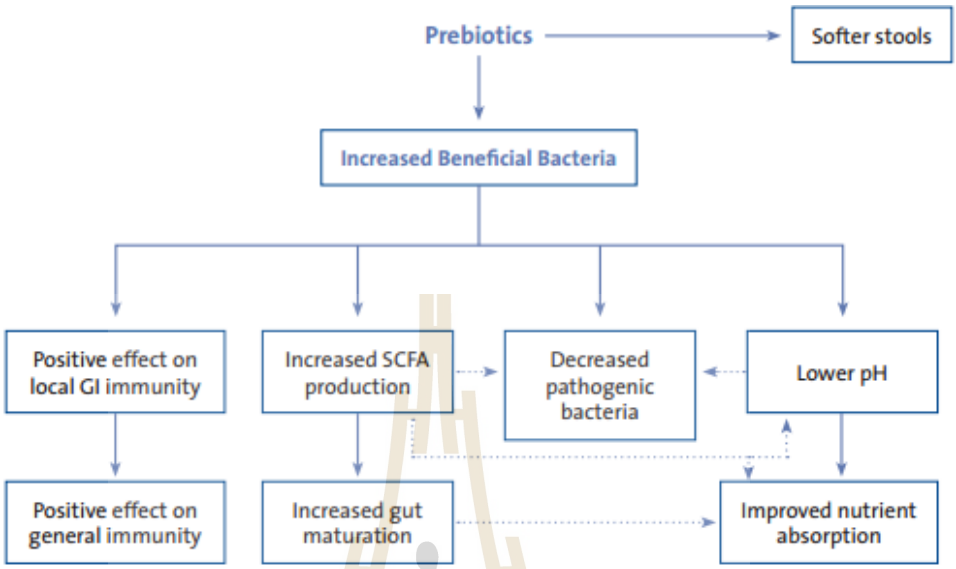

Figure 2.6 Potential Beneficial Effects of Prebiotics on Health

Source : S. Donovan. et al., 2008

The term 'prebiotic' was first defined in 1995 by Gibson and Roberfroid as 'a non-digestible food ingredient that selectively stimulates growth and/or activity of one or a limited number of bacteria in the colon, thereby improving host health'. As research progressed, three criteria were accepted which a food ingredient should fulfil before it can be classified as prebiotic: firstly, it should be non-digestible and resistant to gastric acidity, hydrolysis by intestinal (brush border/pancreatic) digestive enzymes, and gastrointestinal absorption; secondly, it should be fermentable and; thirdly, it should in a selective way stimulate growth and/or metabolic activity of intestinal bacteria that are associated with health and well being.

Prebiotics are a group of bio-molecules grouped together by virtue of their capability to promote the growth and multiplication of specific beneficial gut

microflora. Ban on the use of antibiotics and hormones as feed additives, consumer awareness, strict quality control measures are the driving factors for intense research and development in the areas of functional food, especially the prebiotic oligosaccharides. Although the concept of functional foods has been introduced a long time ago by great ancient Greek philosopher Hippocrates “Let food be thy medicine and medicine be thy food”, scientific evidences started to support the above principle only recently through the application of prebiotics for modulating the gut microflora. The term “Prebiotics” came into light only recently and is coined by Gibson and Roberfroid (1995), who exchanged “pro” to “pre”, which means “before” or “for” (Aida et al., 2009). Prebiotics may be defined as “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and or activity of one or a limited number of bacteria in the colon”. As it matches with certain aspects of dietary fiber, the updated version of prebiotics encompasses “selectively fermented ingredients that allow specific changes, both in the composition and/ or activity in the gastrointestinal microflora that confers benefits upon host well-being and health” (Gibson et al., 2004). Recently, FAO (2007) defined the prebiotic as “A non-viable food component that confers a health benefit on the host associated with modulation of the microbes”. Although the effect of most functional foods targets only one or a limited number of functions, but the prebiotic targets a range of different physiological functions including better gut health, higher mineral absorption, lowering of cholesterol, immune stimulation and pathogen exclusion (Raschka and Deniel, 2005; Roberfroid, 2007). Therefore, the prebiotics are characterized by their non-digestibility at gastric levels, selective stimulation to the beneficial gut microflora, biological origin and obviously without any residue problems.

Prebiotics are dietary short-chain carbohydrates (oligosaccharides). They have beneficial effects on health and growth performance in farm animals, stimulating the growth and/or activity of one or more of beneficial bacteria. The non-digestibility of prebiotics ensures that they can reach the colon and act as an energy source for bacteria, unlike normal sugars, which get digested directly by the host (Gibson GRet al., 1995). As a result the composition and/or the activity of the microbiota are altered, leading to secondary effects such as increased gas production and a drop in pH. Prebiotics can also prevent the adhesion of pathogens to the mucosa, by competing with its sugar receptors and several studies have shown that supplementing feed with various oligosaccharides have led to reduced susceptibility to *Salmonella* and *E. coli* colonization (Iji PA. and Tivey DR., 1998; Patterson JA. and Burkholder KM., 2003).

The most common non-digestible oligosaccharides (NDO), which are used as prebiotics in farm animals, are the following: Mannanoligosaccharides (MOS), Galactooligosaccharides, FOS, Soybeanoligosaccharides, Isomaltooligosaccharides, Xylooligosaccharides, Lactulose, Inulin (Grizard D. and Barthomeuf C., 1999; Vondruskova H. et al., 2010).

Mechanism of action of prebiotic

Mechanisms by which probiotic and prebiotic supplements affect the micro-ecology of the intestinal tract are not well studied, but at least 3 mechanisms have been identified that could have significance in neonatal calves. Antibacterial agents that are produced and secreted by probiotic organisms may have an inhibitory effect on growth of pathogenic microflora. The stimulation of immune responses may also suppress potential pathogens. Finally, specific competition for adhesion receptors to

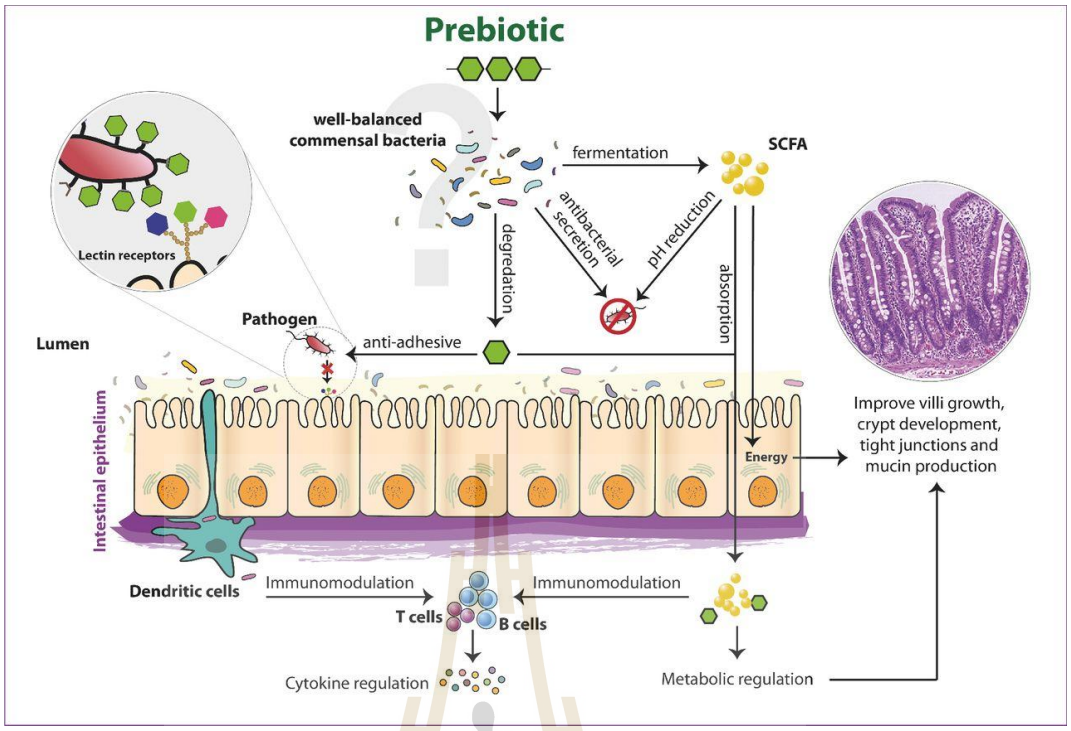

Figure 2.7 Potential mechanisms of action of prebiotics. Prebiotics are metabolized by the gut commensal microbiota. The gut microbiota can ferment prebiotics into SFCA, mainly acetate, propionate and butyrate. SCFA lower the luminal pH, provide energy sources for epithelial cells and have profound effects on inflammation modulators and metabolic regulations. A well-balanced bacterial community can also improve intestinal mucosal structure. Some bacterial strains produce antimicrobial factors or stimulate the immune system by signaling dendritic cells. Oligosaccharides and monosaccharides can reduce pathogen colonization by blocking the receptor sites used by pathogens for attachment to the epithelial cell surface.

Source : Mohsen Pourabedin and Xin Zhao., 2015

the gut epithelium may allow lactic acid bacteria and *Bifidobacteria* to occupy the niche normally required by toxin-producing organisms for colonization. A basic mechanism involved in limiting intestinal pathogens is the competition for nutrients and attachment sites on the intestinal mucosa allowing beneficial bacteria to occupy these attachment sites and increase fermentation and SCFA production. The increased production of short chain fatty acids results in a lower intestinal pH and thereby prevents the proliferation of some disease causing pathogens that are unable to withstand a more acidic environment (Rowland and Gill, 2008).

Unlike the situation with probiotics, where allochthones microorganisms are being introduced into the gut, and have to compete against established colonic communities, prebiotics target the bacteria that are already commensal to the large intestine (MacFarlane et al., 2008). Menneet al. (2000) stated that the key criterion for a prebiotic effect is the demonstration of the selective stimulation of growth of one, or a limited number of potentially beneficial bacteria in the complex fecal microbiota following the consumption of a particular food. Research has indicated that the methods by which prebiotics can exert their effects on the immune system and attenuate inflammation in the colon include increased short chain fatty acid production and increased immunogenic bacteria which contribute to the establishment of a “healthier” microbiota, such as *Lactobacilli* and *Bifidobacteria*. These organisms become predominant and exert possible health-promoting effects at the expense of more harmful species (MacFarlane et al., 2008; Gaggia et al., 2010).

Gibson and Roberfroid (1995) put the conditions for notifying a compound as a prebiotic; it must be indigestible by gastrointestinal enzymes but categorically utilized by selective group of gut beneficial microflora. Unlike other functional foods,

prebiotics exhibit multi-dimensional activities beginning from modulation of gut microflora to mineral absorption, pathogen exclusion etc. (Roberfroid, 2002; Rycroft et al., 2001; Samanta et al., 2010). Therefore, to bring out its positive influence on the livestock system, the channel of activities is supposed to be widely distributed amongst several systems including gastrointestinal, immune regulatory, skeletal organs etc. From the literature it is evidenced that prebiotics are getting its niche for routine consumption for gut health as well as management of gastrointestinal disorders. Obviously most of the studies towards unfolding the mechanism of prebiotic actions are carried out in laboratory animals *i.e.* mice or rats with subsequent support through investigating under *in vitro* or human volunteers (Samanta et al., 2013)

2.8 Immune Development in the ruminant

At birth, the newborn calf has a developing immune system and an underdeveloped digestive system which require proper nutrition and management to maximize development and ensure that the calf has the best possible chance of survival. The main objective during this period is to maximize growth and minimize disease outbreak.

Newborn calves are born agammaglobulinemic, meaning they do not have measureable concentrations of serum immunoglobulins (Ig) critical to their health and survival (Redman, 1979). The calf must obtain Ig within the first 24 hours of life, the period of macromolecular transport. Ig proteins are absorbed through the small intestine into the lymphatic system and further into the blood to become antibodies needed to protect the calf against environmental pathogens (Kruse, 1970). First

milking colostrum is markedly higher in solids, Ig, fat, protein, and vitamins, than compared to whole milk (Foley and Otterby, 1978). When given in sufficient quantities, the high levels of Ig found in colostrum provide passive immunity to the newborn calf enabling it to fight off infections during the first few weeks of life (Redman, 1979).

Colostrum contains 3 major Ig proteins that provide immunity to the calf: IgG, IgA, and IgM. Of the three, IgG is of greatest concern in the first 24 hours of life. Matte et al. (1982) described the process as the entire protein molecule, without digestion altering its form or capabilities, passes through the cells on the mucosal epithelial membrane of the small intestine by pinocytosis and passive transfer into the lymphatic system. Later, the Ig is transferred via the thoracic lymph duct and anterior vena cava to the blood where they become active antibodies (Matte et al., 1982). The IgG then assist in reducing the incidence and severity of many different types of gastrointestinal infections, including Escherichia coli, rotavirus, and Cryptosporidium parvum. In order to assure maximum absorption of IgG, calves must receive 3-4 liters of high quality colostrum within 3-4 hours of birth.

Dairy calves have almost completely developed immune systems at birth, including primary and secondary lymphoid organs and immune cells, because of their long gestation period of 280 days (Halliwell and Gorman, 1989). The first lymphoid organ to develop is the thymus which appears around 40 days post-conception (Schultz et al., 1973; Tizard, 1982). The thymus is responsible for producing thymocytes that mature to become T lymphocytes. Interestingly, the thymus reaches full maturity around 140 days post-conception and steadily decreases in size until regression at puberty (Cortese, 2009; Kushida et al., 2012). At approximately 40 to 45

days, peripheral blood lymphocytes start circulating (Pearson et al., 1976) and starting mid-gestation, peripheral blood lymphocytes can respond to bacterial and viral mitogens (Tizard et al., 1982; Liggitt et al., 1982). Beginning one month prior to birth, the number of peripheral blood T cells decrease from approximately 60% to 30%; however, there are less B cells in the fetus than in mature calves (Chase et al., 2008; Senogles et al., 1979; Kampen et al., 2006).The bone marrow and spleen appear around 55 days post-conception, followed by IgM-carrying cells and lymph nodes 5 days later (Schultz et al., 1973; Tizard, 2013). Bone marrow is a site of leukocyte, erythrocyte, and thrombocyte development and B lymphocyte maturation. Secondary lymphoid organs such as the spleen and lymph nodes facilitate antigen trapping and presentation to lymphocytes. Even though blood lymphocytes and IgM-positive cells appear fairly early in development, antibody production does not start until approximately 130 days with serum IgG and serum IgM (Fennestad and Borg-Petersen, 1962; Tizard, 2013).

Immune status after birth

Despite the neonatal calf's immune system being near complete in development at birth, the number and function of immune cells are altered around the time of birth. This may be due in part to maternal and neonatal glucocorticoids around the time of birth. In addition, immune cell numbers have not yet reached mature levels at birth and the adaptive immune system is naïve in nature.

Suppression of the normal function of innate immune cells, such as chemotaxis and phagocytosis, can last up to four months (Hauser et al., 1986). This decrease in function may be related to the effects of high serum steroids such as cortisol released by the fetal adrenal gland during parturition (Fauci et al., 1976;

Barrington, 2001). A study conducted by Salvemini et al. (1995), found that when the glucocorticoid dexamethasone was administered to rats, iNOS protein expression was inhibited and NO2- was suppressed. Nitric oxide synthase and nitrite both play a role in oxidative burst responses of leukocytes which demonstrates the suppressive potential of glucocorticoids.

As a result of immune suppression at birth, the calves' immune system does not mature until 5 to 8 months after birth. At birth, amounts of circulating complement are less than 20% the amount of mature calves, but increase to 50% by one month of age (Firth et al., 2005; Chase et al., 2008). Adult amounts of complement are not reached until approximately 6 months of age (Cortese, 2009). The number of neutrophils decrease after birth, however the ability of the neutrophils to function increases. Neutrophils are able to respond to pathogens by one week of age, but neutrophil function does not reach full maturity until five months of age (Hauser et al., 1986). Mature amounts of T cells such as CD4+, CD8+, and TCRγδ+ are not reached until approximately 8 months of age (Cahill, 1999; Cortese, 2009). Similarly, B cell amounts increase from 4% of total lymphocytes at 1 week of age to 20% by 6 to 8 weeks of age (Kampen et al., 2006) as well as an increase in circulating IgA and IgG at this time (Husband and Lascelles, 1975).

The acquired immune system is also naïve at birth and relies on exposure to antigens. Additionally, immune responses are biased toward T helper 2 immune responses from placental production of progesterone, prostaglandin E2, and cytokines such as IL-4 and IL-10 which suppresses T helper 1 immune responses in utero (Morein et al., 2002). The immature, naïve, and potentially altered nature of the neonatal immune system exhibits the importance of passive transfer of maternal

immune protection to protect the calf while the immune system becomes fully competent. Windeyer et al. (2014) found that greater than 20% of the BRD cases from 2,874 heifer calves may have been prevented if those calves had not had failure of passive transfer of immunity. Calves that had failure of passive transfer also had lower body weights than calves with successful passive transfer.

2.9 Development of Digestive System

Bethany Leann Fisher. (2011) reported once calves are no longer fed colostrum and transition milk, most producers begin feeding a commercial milk replacer. When a calf consumes colostrum or milk replacer, the liquid feed bypasses the rumen and goes directly into the abomasum via the esophageal groove. It then enters the abomasum which bears many similarities to that of a monogastric stomach. From the abomasum, the remaining components of the milk replacer enter the intestinal tract with the colon being the final stop before excretion.

The concept of the gastrointestinal (GI) tract as an ecosystem is based on the interactions among the resident assemblages of microorganisms, the structural and functional characteristics of the GI tract and the responses to dietary inputs (Buddington, 2009). At birth the neonatal calf's digestive system functions similar to that of a monogastric animal. The GI tract has been described as a physical barrier that is composed of epithelial cells lining the digestive tube, tight junctions that bind them together, and a chemical barrier which consists of secretions that can influence epithelial cells and maintain barrier function (Dubert-Ferrandon et al., 2008). Takahashi and Kiyono (1999) described the intestinal tract as the largest immunological organ of the body, while Brouns et al. (2002) concluded it was the

organ with the greatest surface area and metabolic capacity. The presence of a balanced gut microflora is required for this system to function efficiently.

Mackie et al. (1999) noted that the microbial succession of the GI tract in the first few weeks of life of the preruminant calf is strikingly similar to that of a monogastric newborn. During the birth process and shortly thereafter, microbes from the mother and surrounding environment colonize the GI tract of the infant or neonatal calf. The dairy calf is removed from its mother shortly after birth and fed the mother's colostrum. During this process the calf is in constant contact with sources of bacteria from the environment, handler, and in the colostrum resulting in the initial colonization of the GI tract.

The large intestine is the primary site of microbial colonization and is characterized by large numbers of bacteria and relatively high SCFA concentrations (Mackie et al., 1999). Cummings (1997) stated in the human the colon, along with its bacterial microflora, is an important organ that provides a variety of functions, such as digestion, fermentation, immunological and protective actions, as well as detoxifying functions, which are essential to the whole organism. According to Roberfroid (2008) the gut microflora appears to play important nutritional and physio-pathological roles such as the prevention of gut colonization by potentially pathogenic microorganisms by efficiently outcompeting invading pathogens for ecological niches and metabolic substrates. Callaway et al. (2008) considers the microbial population of the intestinal tract a complex natural resource that can be utilized in an effort to reduce the impact of pathogenic bacteria that affect animal production and efficiency.

Along with modulation of the immune system, the microflora also provides important sources of energy for the cells of the gut wall through fermentation of

carbohydrates to SCFA (Roberfroid, 2008). Macfarlane et al. (2008) found SCFA production to be one of the most important physiological processes mediated by colonic microorganisms. The three main SCFAs produced from this fermentation are acetate, butyrate, and propionate. These SCFAs have been shown to affect colonic epithelial cell transport, colonocyte metabolism, growth and differentiation, provide energy to various organs of the body, and aid in reducing the severity of diarrhea (Cummings, 1995; Cummings and MacFarlane, 1997; Nugent, 2005). Butyrate has been shown to induce cell growth and increase absorptive surface area of the gut and is metabolized by the colonic epithelium, changing the microflora of both the small and large intestines (Cummings and Macfarlane, 1997; Hawthorne and Abrams, 2007). Propionate is shown to improve large bowel circulation thereby stimulating epithelial proliferation and helping to maintain epithelial integrity (Annison and Topping, 1994; Sakata, 1989). While found in the greatest quantities in the colon, acetate exerts its actions on other organs of the body and is used in the formation of butyrate. Increased SCFA concentrations and lowered pH is thought to prevent the overgrowth of pH-sensitive pathogenic bacteria (Topping and Clifton, 2001) allowing proliferation of beneficial bacteria and thereby improving the overall health of the GI system of the animal.

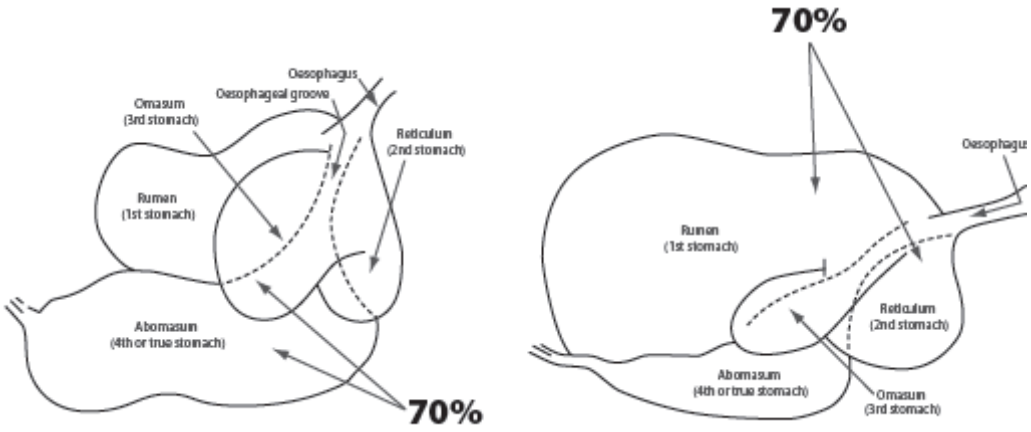

Figure 2.8 The stomach system of a lamb

a) The stomach system of a lamb showing the undeveloped rumen

b) The stomach system of an adult sheep, where the rumen and reticulum make up 70%

Source : Greenwood. (2005)

2.10 Gastrointestinal microflora

Based on the occurrence of microbial habitat in the gastrointestinal tract (Figure 2.9), domestic animals may be classified into two categories viz.; mono gut fermentor- non-ruminants (namely poultry, swine, horse, rabbit, donkey etc) and twine gut fermentors- ruminants and pseudo-ruminants (namely cattle, buffalo, sheep, goat, mithun, camel etc.). The gastrointestinal tract of ruminant is featured with the provision of two microbial habitats i.e. foregut (rumen, reticulum and omasum) and hindgut (caecum). On the other hand non-ruminants are featured with the presence of single microbial habitats i.e. hindgut only. The presence of foregut with enormous capacity to house diverse categories of microflora provide the edge to ruminant animals over the non-ruminants for consumption and dependence on lingo cellulosic biomass to obtain their energy requirements through volatile fatty acids (viz.; acetate,

propionate, butyrate etc.) generated from the degradation and anaerobic fermentation of fibrous materials. The single fermentation site i.e. at the hindgut of non-ruminant animal permeates partial dependence over the volatile fatty acids for energy requirements. It is estimated that rumen fluids contain (number/mL) 10^{10} to 10^{12} bacteria, 10^{4} to 10^{6} protozoa, 10^{8} to 10^{9}archea, 10^{2} to 10^{4} fungi in addition to yeast, phage etc. (Hobson et al., 1988; Hungate, 1966; Samanta et al., 2003).

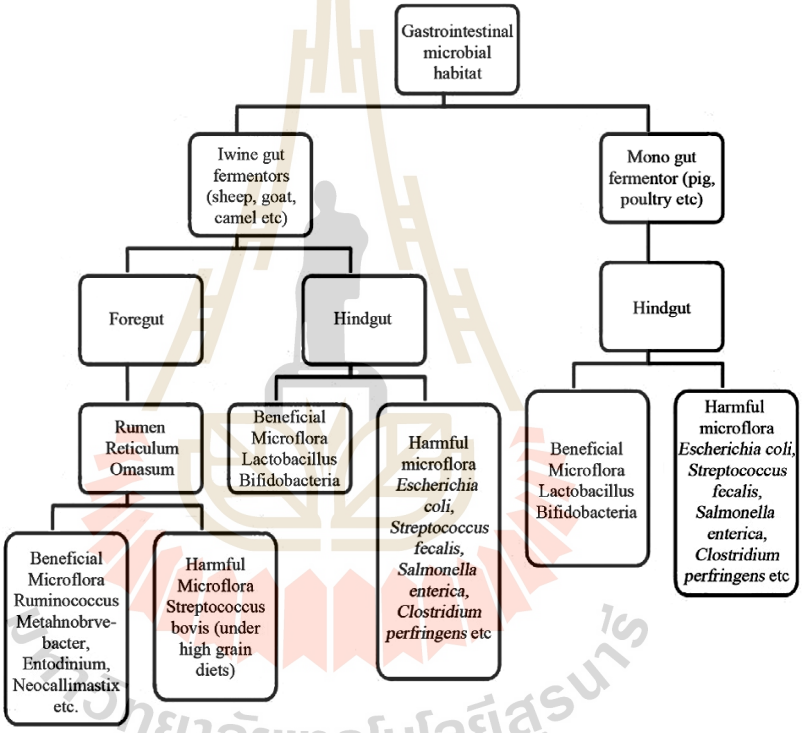

Figure 2.9 Classification of livestock based on microbial habitats.

Source : Samanta et al., 2013

Microflora of the gastrointestinal tract could be broadly grouped into two main categories namely pathogenic or harmful or detrimental microflora and health promoting or friendly or beneficial or pathogen suppressive microflora. The class of pathogenic bacteria includes *E. coli, Streptococcus faecalis, Salmonella enterica,*

C. perfringens etc. *Lactobacillus* and *Bifidobacteria* inhabited in the gastrointestinal tract are presumed to be the major members of beneficial or health promoting class. The significance of prebiotic consumption arises from their ability to selectively stimulate the growth and multiplication of indigenous *Bifidobacteria* and *Lactobacilli* in the hindgut, which in turn suppresses the activity of putrefactive or harmful bacteria for reducing the concentration of toxic fermentation products in the gastrointestinal tract (Samanta et al., 2007, 2010; Tomomatsu, 1994).

2.11 The role of intestinal microflora

A discussion on the impact of the gastrointestinal tract on health and immune status of the calf would not be complete without also covering the role of commensal microorganisms and their symbiotic relationship with the calf. Microflora in the intestine is one of the most densely populated microbial habitats known in the body (Gill et al., 2006). In humans and cattle, it is estimated that there are more than 1014 commensal microbes encompassing more than 400 different species (Ley et al., 2006). The gastrointestinal tract of the calf is devoid of flora at birth, but is colonized shortly after by the fecal and vaginal flora during delivery (Eckburg et al., 2005). The population of microflora colonizing the gastrointestinal tract is influenced by the environment and diet of the calf, the dam, and genetic background (Ozutsumi et al., 2005). Neonatal calves' microbial communities are comprised predominantly of facultative anaerobes from the environment such as *Enterobacteriaceae*, *Streptococcus*, and *Staphylococcus*. However, strict anaerobes, *Bifidobacterium*, *Bacteroides*, *Lactobacilli*, and *Clostridia*, dominate the gastrointestinal tract as the calf ages (Edrington, 2012; Ballou, 2015). Commensal microflora, for example

Lactobacilli and *Bifidobacteria*, form a barrier much like the mucus layer that limits the colonization of pathogenic microorganisms in the gastrointestinal tract. Certain commensal microorganisms may also contribute to the production of mucus and antimicrobial factors (Shahani and Ayebo, 1980). Furthermore, commensal organisms have been demonstrated to stimulate the immune system and growth of gut colonocytes and improve digestion through fermentation (Guarner and Malagelada, 2003).

The health of organisms largely depends on the composition of the intestinal microflora. Its composition and function can be beneficially influenced by many factors. These factors can be significantly supported by probiotics, prebiotics, organic acids, zinc oxide and plant extracts. (Vondruskova H. et al., 2010)

An imbalance of commensal microflora to pathogenic microbes can lead to disease such as diarrhea in calves (Ishihara et al., 2000). This imbalance can be caused by several factors including diet, stress, and the environment (Guarner and Malagelada, 2003). Salmonella is a pathogenic microorganism that can cause fever and diarrhea when it dominates in the normal microbiome (Smith, 2002). Clostridium and *E. coli* are also examples of pathogenic bacteria that can cause harm to calf health.

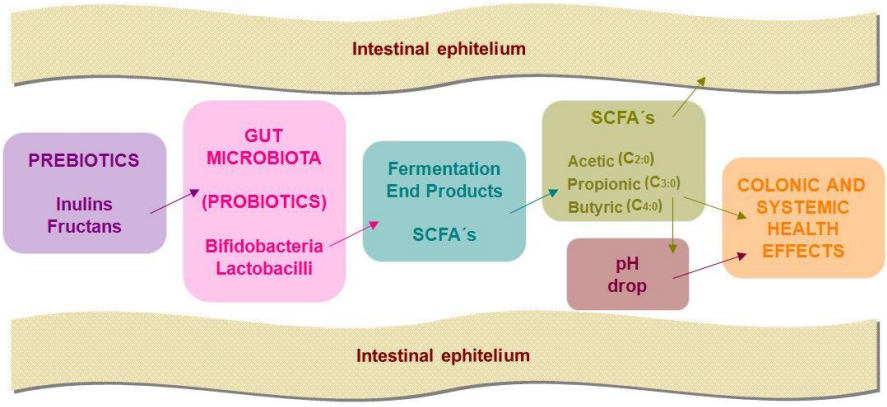

Figure 2.10 General events taken place in the large intestine. Prebiotics are the specific food for probiotics which ferment them to produce short chain fatty acids (SCFAs) to improve the host health.

Source : Alicia Huazano-García and Mercedes G. López. (2013)

2.12 Real-time PCR in the microbiology

The mammalian intestinal tract harbors a complex bacterial ecosystem that has not yet been fully characterized (Jean Marc Delroisse et al., 2008). Molecular techniques introduced in microbial ecology have made it possible to study the composition of intestinal flora in a culture-independent way based on the detection of rRNA genes. Progress in molecular biology has led to the development of alternative, culture-independent methods. One of the most widely used approaches for intestinal microbiota assessment has been to focus on 16S rRNA genes as target molecules. Specific PCR primers can be designed on the basis of the variable regions of this sequence to detect some species or groups of bacteria that are predominant in the gastrointestinal tract (Matsuki et al., 1999 and Matsuki et al., 2002; Wang et al., 1994 and Wang et al., 1996).

The recent development of real-time quantitative PCR methods could lead to accurate knowledge about the composition of gastrointestinal communities. This approach could also provide useful tools for clinical assessment of probiotic and prebiotic efficiency. The TaqMan assay (Applied Biosystems) has been the main assay used for the quantification of several bacterial species and bacterial groups in fecal samples (Malinen et al., 2003; Rinttila et al., 2004; Penders et al., 2005). Most recently, real-time quantitative PCR has been used for the specific detection and quantitation of selected bacteria from fecal DNA (Langendijk P S. et al., 1995).

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CHAPTER III

IN VITRO CULTURES OF INULIN FROM CHICORY ON THE ABUNDANCE OF CALVE FECAL MICROBIAL POPULATIONS BY REAL -TIME PCR

3.1 Abstract

The objective of this study was to the effect of inulin from chicory level on the abundance of bacteria and their fermentation using in vitro of calve fecal samples. Two levels of starch and 4 levels of inulin from chicory were a completely randomized design with a 2 x 4 factorial arrangement of treatments. Factors consisted of starch levels (0 and 1% starch, respectively) and inulin from chicory levels (0, 1, 2 and 4%, respectively). Fresh fecal samples were collected from 3 Jersey calves after feeding the milk. (Approximately 32 days after birth). In the culture, sterile medium + fecal slurry + treatment these culture tubes were carried out in triplicate and incubated 37 ^{o} C for 24, 48 hr in a shaking incubator. Inulin, at present mainly extracted from chicory used in this study was increased the total gas production of fecal fermentation with inulin at the end of the 24 hr was increased linearly when increasing percent of inulin which is simultaneously reflected by the large increase in gas production at high percent of inulin and gas production when the time of incubation increase (from 0 to 24 hr). In contrast, the pH values of the fecal fermentation at 24 hr was decreased linearly ($P<0.001$) with increasing percent of inulin. The volatile fatty acid (VFA) concentrations at the end of the 24 and 48 hr of incubation were influence the levels

of inulin. The proportion of acetate to propionate ratio (A/P) was significantly at 24 and 48 hr of incubation. The addition of starch (0 and 1% starch) have influence acetate, butyrate and A/P ratio ($P<0.001$) at the end of the 24 hr but at the 48 hr did not influence butyrate, valerate and iso-butyrate. Nevertheless, in this study compare with control (0% inulin) significant reduced acetic acid and increased propionic and butyric acid proportions. The addition of 0 or 1% starch did not differ the abundance of *Escherichia coli, Clostridium difficile* at 24 hr and *E. coli* at 48 hr incubation. The abundance of Total bacteria, *Bifidobacterium* and *Lactobacillus* abundance were increased linearly with increasing levels of inulin but abundance of *E. coli*, *C. difficile* were decreased linearly with increasing levels of inulin. In general, the reduction of intestinal pH is a positive outcome, as a more acidic environment might protect against undesirable and pathogenic bacteria. *In vitro* fermentation and animal studies have demonstrated that supplementing the diet with inulin decreased the pH. Inulin did not suppress *E. coli* and *C. difficile* which are harmful microbes cause of diarrhea in the ruminant animals. But supported *Bifidobacterial* and *Lactobacillus* abundance comparable with high levels of inulin. However, some of microorganisms in the rumen can digestion inulin, thereafter the efficiency of inulin in digestive system decreases. But the digestive system of the young goat, and other young ruminants is very similar to that of the pig and human (explaining the term 'preruminant').

Keywords : *in vitro*, inulin, fecal, real - time PCR, ruminant

3.2 Introduction

The current has banned all in-fed use of antibiotic. Therefore, alternative to reduce the use of antibiotic in the animal feed. The use of antibiotics in feed is being considered for elimination (or intense regulation) in other parts of the world. This perspective has stimulated nutritionists and feed manufacturers to search for new, safer alternatives. The primary alternatives studied include acidification of the feed by organic acids, feeding probiotic organisms and feeding prebiotic compounds. (Verdonk J. M. A. J. et al., 2005). Recently, prebiotics has been introduced as a new concept under functional food science owing to concern over residues of antibiotic, consumer awareness and safety features. Keeping in view the above circumstances, presently animal nutrition research draws more attention on feed safety, quality, designer animal products, healthy animal poducts, ecological treatment for digestive disorder and organic animal production (samanta A.K. et al., 2013).

Inulin is carbohydrate form of fructan; it is fiber source which is not digested in digestive system (stomach and intestine) in single stomach animal or human. Therefore, inulin will fall into colon and is benefit to body growth due to an increase in number of health benefit microorganisms, such as *Lactobacillus* and *Bifidobacteria* (Younes et al., 1995; Kaur and Gupta, 2002). Inulin reduce in *E. coli* and *Clostridium* which are dangerous to health, for example, diarrhea, will be determined. Ruminant animals (cattle, buffalo, goat, sheep and deer) some of microorganisms in the rumen can digestion inulin, thereafter the efficiency of inulin in digestive system decreases. However, the digestive system of the young goat and other young ruminants is very similar to that of the pig and human (explaining the term 'preruminant'). During these first stages of milk feeding, the abomasum (true stomach) and small intestine play a

relatively important role with respect to digestion and nutrition. In young goats, and other young ruminants, the suckling reflex triggers the esophageal groove to close so that milk bypasses the rumen and flows directly in to the abomasum where clotting and some digestion occurs. Milk protein is rapidly digested in the small intestine, as is lactose (Anita O'Brien., 1998).

Although several studies have presented the inulin effects of animal feed and using *in vitro* fermentation of rumen fluid samples, no study has been reported that fermentation used *in vitro* culture of calve fecal samples. The objective of this study was to analyses the effect of inulin on the abundance of bacteria and their fermentation using *in vitro* of calve fecal samples.

3.3 Objective

To study the effect of inulin from chicory level on the abundance of bacteria and their fermentation using *in vitro* of calve fecal samples.

3.4 Materials and methods

3.4.1 Inulin

Purchased the Inulin of chicory roots (Prebiofeed 88) for animal applications from SOCODE S.C. The study was a completely randomized design with a 2 x 4 factorial arrangement of treatments. Factors consisted of starch concentration (0% starch and 1% starch, respectively) and inulin from chicory concentration (0%, 1%, 2% and 4%, respectively).

3.4.2 Animals and fecal sample

The sample fecal collected from three Jersey calves with average live weights of 93 ± 7 kg about 32 day of age. Fecal was collected from each of three calves into a plastic bag using a sterile tongue depressor, then placed in an insulated cooler bag containing frozen on ice. The samples were transferred to the laboratory within 10 min. The fecal sample was immediately transferred into a sterile 100-ml bottle (10%, w/v) in phosphate-buffered saline (PBS) and glass bead-containing using a sterile tongue depressor and then placed into an anaerobic chamber containing N_2 (95%), H_2 (3%) and CO_2 (2%). Equal volumes of the fecal collected from each of the calves were combined as the inoculum.

3.4.3 Fecal batch culture fermentation

Inulin from chicory were weigh into the sterile Hungate tube of treatment 1 (no 1% strach): 3 levels; 1% inulin, 2% inulin, 4% inulin and treatment 2 (have 1% strach): 3 levels; 1% strach + 1% inulin, 1% strach + 2% inulin, 1% strach + 4% inulin, respectively. The control cultures received no inulin. The sterile medium was made anaerobic in the chamber and dispensed into Hungate tube (9 ml/tube). The basal medium contained (in g/l): peptone, 2.0; yeast extract, 2.0; NaCl, 0.1; K_2HPO_4, 0.04; KH_2PO_4, 0.04; $MgSO_4.7H_2O$, 0.01; $CaCl_2.2H_2O$, 0.01; $NaHCO_3$, 2.0; haemin, 0.005; L-cysteine hydrochloride, 0.5; bile salts, 0.5; The following liquid addition were made: tween 80, 2.0 ml; vitamin K, 10 µl; 0.025% (w/v) resazurin solution, 4.0 ml (Vernazza CL. et al., 2006; Olano-Martin E. et al., 2000; Sanz ML. et al 2005; Stiverson J. et al., 2014). The initial pH of the culture medium was 7.0. The samples were inoculated with 1 ml of fecal slurry, which was prepared by homogenizing fresh Jersey calves fecal (10%, w/v) in phosphate-buffered saline (PBS (in g/l) NaCl, 8.0;

KCl, 0.2; Na_2HPO_4, 1.15; KH_2HPO_4, 0.2; pH 7.0). Each tube was added (1 ml) of fecal slurry, was mixed well by vortexing vigorously. All Hungate tube was maintained under anaerobic conditions with O_2-free N_2. After sealed with butyl rubbers plus crimped aluminum seals, these culture tubes were carried out in triplicate and incubated 37 ^{o}C for 24, 48 hr in a shaking incubator. Then, 2 ml of liquid samples were individually collected into microcentrifuge tubes and centrifuged at 4 ^{o}C for 10 min at 16,000 xg and the peletted was preserved at -80°C for microbial analysis and supernatant aliquot into 2 microcentrifuge tubes, which was preserved at -20°C for VFA analysis using gas chromatography. The pH values of the *in vitro* cultures were recorded using a pH meter. Gas pressure in each the culture Hungate tube was measured using a manometer (Traceable; Fisher Scientific) to determine total gas production.

3.4.4 Microbial community DNA extraction

The samples were thawed in 4 ^{o}C overnight. Total community DNA was extract using the RBB+C method (Yu and Morrison, 2004), using the repeated bead-beating and column purification method using the QIAamp DNA Stool Mini kit. The DNA quality was evaluated by agarose gel (1.0%) electrophoresis. The DNA concentrations were quantified using a Nano-Drop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE). Aliquot the DNA solution into two tubes of 1.5 ml Eppendorf tubes: One as backup stored at -80 ^{o}C and the other one stored at -20 ^{o}C until analysis.

Table 3.1 Primer used for real-time PCR quantification.

Primer	**Sequence (5' -3')**	**Targeted bacteria**	**Annealing temperature**	**Amplicon length (bp)**	**Reference**
27f	AGA GTT TGA TCM TGG CTC AG	Bacteria	54 ^{o}C	1535	Lane DJ, 1991
1525r	AAG GAG GTG WTC CAR CC				
340f	TCC TAC GGG AGG CAG CAG T	Bacteria	60 ^{o}C	467	Nadkarni MA., et al, 2002
806r	GGA CTA CCA GGG TAT CTA ATC CTG TT				
Bif164-f	GGG TGG TAA TGC CGG ATG	*Bifidobacteria*	60 ^{o}C	530	Satokari RM., et al, 2001
Bif662-r	CCA CCG TTA CAC CGG GAA				
Cdif-706f	ATT AGG AGG AAC ACC AGT TG	*C. difficile*	54 ^{o}C	307	Xia Q., et al, 2012
Cdif-994r	AGG AGA TGT CAT TGG GAT GT				
ECA75F	GGA AGA AGC TTG CTT CTT TGCT GAC	*E. coli*	56 ^{o}C	545	Sabat G., et al 2000
ECR619R	AGC CCG GGG ATT TCA CAT CTG ACT TA				
Lac1	AGC AGT AGG GAA TCT TCC A	*Lactobacillus*	60 ^{o}C	345	Sabat G., et al 2000
Lac2	ATT YCA CCG CTA CAC ATG				
InvA-F	GAA ATT ATY GCC ACR TTC GGG CAA	*Salmonella*	52 ^{o}C	282	Shan Wei., et al, 2013
InvA-R	TCA TCG CAC CGT CAA ARG ARC C				
*****TaqMan probe	6-FAM-5'-CGT ATT ACC GCG GCT GCT GGC AC-3'-TAMRA				Nadkarni MA., et al, 2002

*Taqman probe to be used with primers 340f and 806r for quantification of total bacteria

3.4.5 Preparation of sample-derived real-time PCR standards.

The DNA standards for the real-time PCR assays were prepared as described (Chen J. et al., 2007). Briefly, for each of the real-time PCR assays, 1-sample-derived standard specific primer was used the general of samples from a pooled of all DNA sample in experiment an equal amount of concentration of DNA from all the samples to be analyzed. For total bacteria, the standard was prepared used the universal primers 27f and 1525r (Lane, 1991), which amplify nearly full-length *rrs* genes from most bacteria. The specific primer was PCR product used pool of DNA sample, for each PCR reaction in 50 ul volumes, which contains, 1X standard Taq Reaction buffer (10 mMTris-HCl, 50 mMKCl, and 1.5 mM $MgCl_2$ (pH8.3)), 200 uMdNTP, 500 nM of each primer, 670 ng/ul bovine serum albumin, 1.25 U of Taq DNA polymerase (BioLabs) and DNA template. The PCR assay using a PTC-100 thermocycler (MJ Research, Waltham, MA). The PCR thermal program consisted of an initial denaturation step at 94 ^{o}C for 5 min; 40 cycles of a 30 s denaturation step at 95^{o}C, a 30 s annealing step at respective temperature in table 1, and a 40 s elongation step at 72 ^{o}C; and a final extension step at 72 ^{o}C for 7 min before a 4 ^{o}C hold. The targeted sequences in the samples were confirmed by agarose gel (1.0%) electrophoresis. No template control and positive control samples were included in all the PCR assays. And each primer of PCR product was purified by a Qiagen PCR Product Purification kit (Invitrogen). The DNA concentration of each qPCR standard was determined using the Quant-iT kit (Invitrogen Corporation, Carlsbed, CA, USA) and an Mx3000P real-time PCR system (Stratagene, La Jolla, CA). The copy number of DNA concentrations were calculated on the basis of the length of the PCR product and the mass concentrations each standard. The standards were stored at -80 ^{o}C. Tenfold

serial dilutions (1:10) of standards were made before each real-time PCR assay by using TE buffer.

3.4.6 Quantification of abundance of bacteria by quantitative real-time PCR (qPCR) analyses.

The primers and probes used in the real time PCR assays were shown in the table 3.1. The primer sequences were compared with database sequences by BLAST search (GenBank). The *Salmonella* were not quantified because initial endpoint PCR analysis of the standard showed very low occurrence of this group of bacteria (data not shown). The population sizes of total bacteria were quantified using a TaqMan probes assay, while those of another specific primers (i.e., *Bifidobacteria*, *C. difficile*, *E. coli* and *Lactobacillus*) were quantified using SYBR-based. The real-time PCR assays were performed using an Mx3000P real-time PCR system (Stratagene) for each real-time PCR in 25 uL reaction volumes in triplicate. The real-time PCR master mix contained, 1XPCR buffer, 1.75 mmol/L $MgCl_2$, 670 ng/ul bovine serum albumin, 200 umol/L dNTP, 500 nmol/L each primer, 0.133x of SYBR Green I (Invitrogen), 30 nM reference dry ROX (Stratagene), 1.25 U Platinum Taq DNA polymerase (Invitrogen) and added 0.5 ul of DNA. Total bacteria were quantified using real-time PCR the contained, 1XPCR buffer, 1.75 mmol/L $MgCl_2$, 670 ng/ul bovine serum albumin, 200 umol/L dNTP, 500 nmol/L each primer, Taqman probe, 1.25 U Platinum Taq DNA polymerase (Invitrogen) and added 0.5 ul of DNA.

The thermos profiles of total bacteria were quantified using real-time PCR as described previously by Nadkarni et al. (2002). The thermos profiles of the real-time PCR assays for *bifidobacteria*, *C. difficile*, *E. coli* and *lactobacillus* were as follows : initial denaturation at 95 ^{o}C for 4 minutes, followed by 45 cycles of denaturation at 94

^{o}C for 30 seconds, annealing for 30 seconds at the respective annealing temperature in the table 1, elongation at 72^{o}C for 40 seconds, and 86 ^{o}C for 18 seconds. After 45 cycles, were held at 95 ^{o}C for 1 minute, cooled down to 55 ^{o}C for 30 seconds, and slowly ramped up to 95 ^{o}C, were held for 30 seconds to terminate the reactions. Fluorescent signals were acquired twice per cycle: at 72 ^{o}C and 86 ^{o}C (endpoint), as well as during ramping from 55 ^{o}C to 95 ^{o}C (all point).

3.4.7 Analysis of volatile fatty acid

All the fermentation samples were quantitatively determined for volatile fatty acid (acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid and valeric acid) using gas chromatography was a Hewlett-Packard 5890 A, as described by Timothy J. et al., 2013. Briefly, The temperature of oven was initially 113°C and increased 2°C/min over 14 min; temperature of the injector was 150°C and the temperature of the detector was 180 ^{o}C, 1.0 µl of the sample was injected in the column is from Supelco, 6-ft by 2 mm glass, and packed with GP 15% SP-1220/1% H_3PO_4 stationary phase on 100/120 Chromosorb W AW support, using N_2 as carrier gas (20 ml/min). Detector is of flame ionization type and fueled by H2 (300 ml/min) and air (300 to 400 ml). The levels of VFA were determined using 2-ethylbutyric acid as an internal standard. After every ten samples the column was cleanred by injection of 1.0 µl standard VFA mix (14.12 mM acetic acid, 4.71 mM propionic acid, 0.471 mMiso-butyric acid, 3.53 mM butyric acid, 0.706 mMiso-valeric acid, 0.706 Mm valerate and 10 mM 2-ethylbutryate)

3.4.8 Statistical analysis

The data obtained on fecal fermentation characteristic were analyzed using the PROC GLM procedure of SAS program in a 2 (0% starch and 1% starch) x 4 (levels

of 0% inulin, 1% inulin, 2% inulin, 4% inulin) factorial in CRD design. To compare the inulin effects among levels within each starch to test the levels effects. Orthogonal polynomial contrasts with control were used to analyze linear, quadratic and cubic effects of the increasing levels of inulin. The abundance from each real-time PCR assay were converted to average estimate of rrs gene copies per ml of fecal fermentation sample and log-transformed before statistical analyses to improve normality. Significance was declared at $P \leq 0.05$, tendency at $0.05 < P \leq 0.10$, whereas numerical changes at $0.10 < P \leq 0.15$.

3.4.9 Experimental location

The experiment was conducted at Waterman Dairy Unit,Columbus, OH, the center for Scientific and Technological Equipment, Animal Science Building,The Ohio State University, USA.

3.5 Results and discussion

3.5.1 Total gas and pH at the 24 hr.

The initial pH of the culture medium was approximately 7.0. In table 3.2 show the total gas and pH values of inulin from chicory powder in the fecal fermentation. The total gas production of fecal fermentation with inulin at the end of the 24 hr was increased linearly when increasing percent of inulin which is simultaneously reflected by the large increase in gas production at high percent of inulin and gas production when the time of incubation increase (from 0 to 24 hr). Philippeau C. (2010) studied about effects of FOS in veal calf ileal contents *in vitro*. Addition of scFOS increased gas production when the time increase (from 4.5 to 9.0 hr), while the concentration of scFOS only affected ($P<0.05$) gas production at 7.5 and 9.0 hr of incubation.

In contrast, the pH values of the fecal fermentation at 24 hr was decreased linearly ($P<0.001$) with increasing percent of inulin. This study but similar to that reported by Ning Zhang et al. (2013) who studied some members of the fecal bacteria utilized garlic fructans to grow resulting in the pH of the cultures *in vitro*. The pH changes at different incubation periods during the 24 hr *in vitro* batch fermentation. The pH values of the garlic fructans cultures decreased significantly with time ($P<0.05$), and at 24 hr, the pH were 4.67 (garlic fructans, DP: 16) and 4.97 (garlic fructans, DP: 21). This was likely a result of the production of organic acids, such as short chain fatty acids. This is consistent with findings by Stiverson J. et al. (2014) who studied prebiotic products using *in vitro* cultures of infant fecal samples reported the pH of the initial cultures prior to incubation was approximately 6.4. The addition of the GOS product or both the GOS product and the inulin product (Beneo® HP) decreased the culture pH by lower than 2 pH units after 12 hr of incubation. Louise Kristine Vigsnaes et al. (2011) who studied about fermentation of sugar beet Arabino-Oligosaccharides by fecal microbiota obtained from patients. Reportedfor all types of samples, the pH was lower in samples incubated with FOS and AOS than in the samples incubated without added substrate. This is consistent with findings by Pinna C. et al. (2014) studies *in vitro* effect of some prebiotic substances and 2 dietary protein levels on the composition and activity of feline fecal microbiota. The pH values after 6 hr. of incubation was reduced ($P<0.05$) by gluconic acid (-0.09) and FOS (-0.15) versus the control. At 24 hr., the pH was lower than in the control ($P<0.05$) in all treated samples (-0.28 for gluconic acid, -0.11 for carrot fiber, -0.43 for FOS, -0.26 for GOS, -0.48 for Lactitol (LAC) and -0.52 for pectins from citrus fruit). Pinna C. et al. (2016) present *in vitro* study investigated whether the utilization

of FOS may influence canine fecal microbial population in presence of diets differing in their protein content and digestibility. Values of pH were reduced by FOS at 6 and 24 hr. ($P<0.001$).

Fermentation of carbohydrates by bacteria results in the production of VFA and lactic acid, which reduce intestinal pH (Pinna et al., 2014). In general, the reduction of intestinal pH is a positive outcome, as a more acidic environment might protect against undesirable and pathogenic bacteria (McQuaid, 2005). The low molecular weight oligosaccharides, such as scFOS, can be fermented by veal calves ileal microbiota even when calves were not adapted to their supplementation.

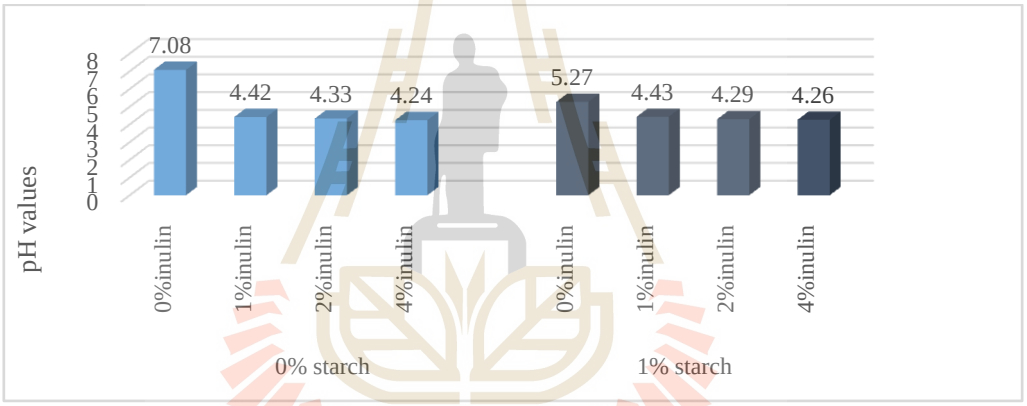

Figure 3.1 Effects of inulin from Chicory powder on pH values in the fecal cultures at the 24 hr.

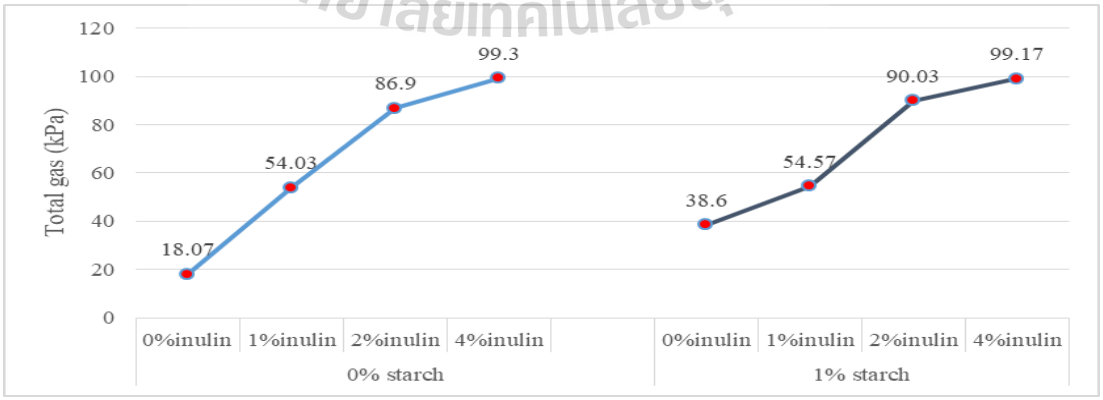

Figure 3.2 Effects of inulin from Chicory powder on total gas in the fecal cultures at the 24 hr.

Table 3.2 Effects of inulin from Chicory powder on Total gas and pH in the fecal cultures at the 24 hr.

Treatment A	Treatment B	pH values	Total gas (kPa)
0% starch	0% inulin	7.08±0.01	18.07±1.17
	1% inulin	4.42±0.02	54.03±3.50
	2% inulin	4.33±0.01	86.90±2.98
	4% inulin	4.24±0.01	99.30±0.87
Contrast			
linear		<.0001	<.0001
Quadratic		<.0001	<.0001
Cubic		<.0001	0.0032
1% starch	0% inulin	5.27±0.09	38.60±1.73
	1% inulin	4.43±0.03	54.57±0.60
	2% inulin	4.29±0.06	90.03±1.62
	4% inulin	4.26±0.00	99.17±1.10
Contrast			
linear		<.0001	<.0001
Quadratic		<.0001	0.0077
Cubic		<.0001	<.0001
SEM		0.00035	0.7776
Effect			
Starch*Inulin		<.0001	<.0001
Starch		<.0001	<.0001
Inulin		<.0001	<.0001

3.5.2 Volatile fatty acid (VFA) concentrations

The cumulative gas production technique can be used to study the effect of inocula (as source of microflora) and substrates on *in vitro* fermentation

characteristics between inocula and substrate (Williams et al., 2001). The cumulative gas production measured fermentation kinetics and end products, such as total gas production, and VFA were also determined. (Shim et al., 2007). Bauer et al. (2004) reported that fecal inocula can be used for *in vitro* assessment of large intestinal fermentation.

The inulin supplementations showed effects on the concentrations of acetic, butyric, propionic, isobutyric, valeric, and iso-valeric acids were analyzed for all the fecal cultures samples (Table 3.3 and Table 3.4).The addition of starch (0 and 1% starch) have influence acetate, butyrate and A/P ratio ($P<0.001$) at the end of the 24 hr but at the 48 hr did not influence butyrate, valerate and iso-butyrate. The VFA concentrations at the end of the 24 and 48 hr of incubation were influence the levels of inulin. The proportion of A/P ratio was significantly at 24 and 48 hr of incubation.

Similar to that reported by Wang W. S. et al. (2010) studies *in vitro* of fiber ferment ability by swine fecal microflora, which after 24 hr *in vitro* fermentation, inulin a lower ($P<0.05$) pH value and VFA concentration as compared with carboxy methyl cellulose (CMC) pH has a strong negative relationship ($r = - 0.94$, $P=0.02$) with total VFA concentrations of the inoculum. *In vitro* fermentation and animal studies have demonstrated that supplementing the diet with inulin decreased the pH with the increased size of cecal VFA (Zdunczyk et al., 2006). Carbohydrates reaching the large intestines may be broken down by the bacterial flora, through fermentation process to produce VFA and other gases such as hydrogen and carbon dioxide (Tomlin et al., 1986).

Nevertheless, in this studies compare with control (0% inulin) significant reduced acetic acid and increased propionic and butyric acid proportions. This is

consistent with findings by Pinna C. et al. (2014) studies *in vitro* effect of some prebiotic substances and 2 dietary protein levels on the composition and activity of feline fecal microbiota, concentrations of VFA after 24 hr of incubation were greater ($P<0.05$) than in the control bottles containing carrot fiber (+41%). Acetic acid proportion was reduced ($P<0.05$) by FOS(-13%), GOS(-12%) and LAC(-17%). The proportion of propionic acid was increased ($P<0.05$) by LAC(+8%). Compared with the control, the proportion of *n*-butyric acid was increased ($P<0.05$) by FOS(+13%), GOS(+15%) and LAC(+10%), whereas the acetic to propionic acid ratio was reduced by LAC (-51%; $P<0.05$). Pinna C. et al. (2016) present *in vitro* study investigated whether the utilization of FOS may influence canine fecal microbial population in presence of diets differing in their protein content and digestibility. Concentration of VFA after 24 hr. of the fermentation, FOS resulted ($P<0.001$) in higher concentrations of total VFA (+43%), acetic acid (+14%), propionic acid (+75%) and *n*-butyric acid (+372%). Both A/P ratio was reduced by FOS ($P<0.01$). The generally accepted that gastrointestinal short-chain fatty acids (SCFAs) acetic, propionic and butyric acid are mostly derived from carbohydrates, while iso-butyric and iso-valeric acids are from protein sources (Maria E. C. et al., 2005). The major end products of carbohydrates fermentation in the large intestine are VFA, CH_4, H_2, CO_2 and NH_3. OF influences the microbiota composition and activity and by the production of VFA it may beneficially affect the gut ecology and health. (Houdijk et al., 1997; Estrada et al., 2001; He et al., Xu et al., 2002). The production of VFA which are produced as end products of the fermentation by microbiota may be beneficial for gastrointestinal tract health (Cummings and Macfarlane, 1991). The individual VFA produced in the large intestine may have a specific role. For instance, acetic acid acts as an energy sources

for muscle tissue and propionic acid is converted in the liver. In addition, butyric acid is the preferred substrate for the colonic epithelial cells (Roediger, 1980). Shim et al., (2007) presented results show that type of fermentable carbohydrate is important in determining the amount and profile of VFA in the large intestine.

Table 3.3 Effects of inulin from Chicory powder on VFA concentrations in the fecal cultures at 24 hr.

Treatment A	Treatment B	Total VFA (mM)	Acetate (mM)	Propionate (mM)	Butyrate (mM)	Valerate (mM)	Isobutyrate (mM)	Isovalerate (mM)	Acetate/ Propionate
0% starch	0% inulin	33.27±1.99	18.36±1.07	6.44±0.43	3.23±0.20	2.55±0.10	1.00±0.07	1.68±0.12	2.85±0.02
	1% inulin	83.42±4.61	31.30±1.15	38.45±1.85	6.84±0.89	5.47±0.77	0.65±0.07	0.71±0.02	0.81±0.01
	2% inulin	79.32±0.36	14.47±0.13	17.65±0.56	24.53±1.08	20.22±0.20	1.94±0.16	0.51±0.00	0.82±0.02
	4% inulin	91.17±0.33	17.22±0.49	17.37±0.66	30.75±1.48	20.75±0.20	4.67±0.21	0.41±0.01	0.99±0.01
Contrast									
Linear		<.0001	<.0001	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001
Quadratic		<.0001	<.0001	<.0001	0.0122	0.0010	<.0001	<.0001	<.0001
Cubic		<.0001	<.0001	<.0001	<.0001	<.0001	0.7900	0.0004	<.0001
1% starch	0% inulin	55.48±2.11	33.45±1.65	8.49±0.09	8.15±0.42	3.02±0.06	0.91±0.00	1.46±0.02	3.94±0.19
	1% inulin	80.34±1.05	32.14±0.76	33.75±0.14	7.55±0.62	5.53±0.45	0.68±0.16	0.69±0.06	0.95±0.03
	2% inulin	78.83±2.79	15.12±0.87	16.51±0.87	24.98±0.56	19.83±0.44	1.90±0.10	0.49±0.01	0.92±0.02
	4% inulin	90.79±3.41	17.92±1.92	16.95±0.26	31.30±0.26	20.94±0.60	4.71±0.62	0.42±0.00	1.01±0.11
Contrast									
Linear		<.0001	<.0001	0.0055	<.0001	<.0001	<.0001	<.0001	<.0001
Quadratic		0.0014	0.0110	<.0001	<.0001	0.0174	<.0001	<.0001	<.0001
Cubic		<.0001	<.0001	<.0001	<.0001	<.0001	0.8506	0.0049	<.0001
SEM		1.3971	0.3014	0.1507	0.1006	0.0409	0.0137	0.0005	0.0013
Effect									
Starch*Inulin		<.0001	<.0001	0.0001	0.0002	0.4866	0.9696	0.0055	<.0001
Starch		0.0018	<.0001	0.0162	<.0001	0.6703	0.9024	0.0123	<.0001
Inulin		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Table 3.4 Effects of inulin from Chicory powder on VFA concentrations in the fecal cultures at 48 hr.

Treatment A	Treatment B	Total VFA (mM)	Acetate (mM)	Propionate (mM)	Butyrate (mM)	Valerate (mM)	Isobutyrate (mM)	Isovalerate (mM)	Acetate/ Propionate
0% starch	0% inulin	35.19±1.56	19.59±0.91	6.61±0.25	3.35±0.12	2.88±.18	1.05±0.06	1.70±0.05	2.96±0.03
	1% inulin	83.06±1.62	31.75±0.82	35.11±1.38	8.69±0.83	8.05±0.62	0.40±0.04	0.70±0.01	0.90±0.02
	2% inulin	90.47±1.37	10.97±0.14	15.04±0.24	33.64±1.45	29.42±0.28	0.90±0.02	0.50±0.01	0.73±0.00
	4% inulin	105.80±6.31	19.41±1.43	13.61±0.70	40.35±2.12	27.74±1.98	4.27±0.06	0.42±0.01	1.43±0.03
Contrast									
Linear		<.0001	<.0001	0.6686	<.0001	<.0001	<.0001	<.0001	<.0001
Quadratic		<.0001	0.0039	<.0001	0.6481	0.0051	<.0001	<.0001	<.0001
Cubic		0.0024	<.0001	<.0001	<.0001	<.0001	0.0593	<.0001	<.0001
1% starch	0% inulin	59.52±1.15	36.45±0.94	6.06±0.09	12.57±0.23	2.76±0.03	0.56±0.01	1.11±0.01	6.01±0.11
	1% inulin	93.56±0.71	38.82±0.75	31.41±0.61	12.38±1.13	9.80±0.92	0.46±0.02	0.69±0.00	1.24±0.00
	2% inulin	95.82±6.56	11.56±0.64	15.26±0.82	37.18±3.79	30.55±2.77	0.80±0.13	0.48±0.01	0.76±0.06
	4% inulin	110.32±5.36	18.53±0.53	14.95±0.59	43.07±3.79	29.57±2.37	3.79±0.72	0.41±0.04	1.24±0.09
Contrast									
Linear		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Quadratic		0.0019	0.0005	<.0001	0.0474	0.0011	<.0001	<.0001	<.0001
Cubic		0.0025	<.0001	<.0001	<.0001	<.0001	0.0196	0.2695	<.0001
SEM		3.3320	0.1374	0.1082	1.0502	0.5073	0.0171	0.0001	0.0008
Effect									
Starch*Inulin		0.0052	<.0001	0.0008	0.1112	0.7178	0.3574	<.0001	<.0001
Starch		<.0001	<.0001	0.0578	0.0004	0.1256	0.0741	<.0001	<.0001
Inulin		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Table 3.5 Effect of inulin of fecal fermentation at 24 hr on abundance of Total bacteria, *E. coli*, *C. difficile*, *Bifidobacterium* and *Lactobacillus* quantified by real-time PCR.

Treatment A	**Treatment B**	**Abundance (log_{10} no. of copies of rrs gene/ml)**				
		Total bacteria	***E. coli***	***C. difficile***	***Bifidobacterium***	***Lactobacillus***
0% starch	0% inulin	9.85±0.07	8.82±0.08	6.23±0.13	7.33±0.03	8.11±0.03
	1% inulin	10.65±0.15	8.40±0.34	6.10±0.12	8.25±0.28	8.63±0.10
	2% inulin	10.58±0.07	8.06±0.00	5.74±0.01	9.08±0.01	9.21±0.00
	4% inulin	10.75±0.12	8.38±0.12	5.84±0.02	9.17±0.14	9.77±0.09
Contrast						
Linear		<.0001	0.0012	0.0006	0.0001	<.0001
Quadratic		0.0004	0.0013	0.1520	0.1267	0.7836
Cubic		0.0028	0.1669	0.0653	0.5601	0.8345
1% starch	0% inulin	10.38±0.13	8.63±0.03	6.00±0.11	9.44±0.03	8.33±0.13
	1% inulin	10.55±0.13	8.47±0.10	6.22±0.07	8.88±0.01	8.59±0.18
	2% inulin	10.59±0.07	8.32±0.07	5.75±0.06	8.75±0.10	8.74±0.08
	4% inulin	10.80±0.06	8.45±0.02	5.91±0.22	8.91±0.07	9.67±0.04
Contrast						
linear		0.0003	0.0777	0.0336	0.1759	<.0001
Quadratic		0.7028	0.1022	0.6587	0.2068	<.0001
Cubic		0.2817	0.4940	0.0007	0.9001	0.0057
SEM		0.0023	0.0042	0.0029	0.0036	0.0022
Effect						
Starch*Inulin		0.0008	0.1246	0.0959	0.0015	0.0018
Starch		0.0182	0.4232	0.8770	0.0126	0.0579
Inulin		<.0001	0.0003	0.0001	0.0920	<.0001

Table 3.6 Effect of inulin of fecal fermentation at 48 hr on abundance of Total bacteria, *E. coli*, *C. difficile*, *Bifidobacterium* and *Lactobacillus* quantified by real-time PCR.

Treatment A	**Treatment B**	**Abundance (log_{10} no. of copies of rrs gene/ml)**				
		Total bacteria	***E. coli***	***C. difficile***	***Bifidobacterium***	***Lactobacillus***
0% starch	0% inulin	9.69±0.07	8.52±0.18	6.23±0.19	7.20±0.10	7.74±0.15
	1% inulin	10.60±0.08	8.44±0.05	5.67±0.06	8.79±0.03	8.82±0.06
	2% inulin	10.71±0.08	8.34±0.06	5.73±0.08	9.14±0.11	9.11±0.08
	4% inulin	10.75±0.11	8.42±0.03	5.90±0.07	9.33±0.03	9.40±0.02
Contrast						
linear		<.0001	0.1048	0.0022	<.0001	<.0001
Quadratic		<.0001	0.1664	<.0001	<.0001	<.0001
Cubic		0.0016	0.3691	0.0689	0.0003	0.0041
1% starch	0% inulin	10.38±0.08	8.52±0.03	6.53±0.02	9.87±0.08	8.20±0.04
	1% inulin	10.65±0.01	8.43±0.07	6.00±0.06	9.08±0.12	8.98±0.06
	2% inulin	10.73±0.05	8.48±0.06	5.84±0.03	9.27±0.04	9.04±0.06
	4% inulin	10.76±0.06	8.46±0.04	5.89±0.05	9.26±0.06	9.37±0.07
Contrast						
linear		<.0001	0.5617	<.0001	<.0001	<.0001
Quadratic		0.0093	0.5236	<.0001	<.0001	0.0002
Cubic		0.4518	0.3450	0.4656	<.0001	0.0002
SEM		0.0010	0.0013	0.0016	0.0013	0.0013
Effect						
Starch*Inulin		<.0001	0.4317	0.0271	<.0001	0.0003
Starch		<.0001	0.2119	0.0003	<.0001	0.0024
Inulin		<.0001	0.1898	<.0001	<.0001	<.0001

3.5.3 Abundance of Total bacteria, *E. coli, C. difficile, Bifidobacterium* and *Lactobacillus*.

The abundance of Total bacteria, *E. coli, C. difficile, Bifidobacterium* and *Lactobacillus* was determined for each inoculum collected from fecal cultures used to determine the effects of the different of % inulin. The starch and inulin did not affect the abundance of *E. coli* at 48 hr (table 3.6). The starch did not affect the abundance of *E. coli* and *C. difficile* at 24 hr and abundance of *E. coli* at 48 hr, but inulin have affect to the abundance of *E. coli* and *C. difficile* at 24 hr. The addition of 0% starch or 1% starch did not differ the abundance of *E. coli, C. difficile* at 24 hr and *E. coli* at 48 hr incubation. The Total bacteria, *Bifidobacterium and Lactobacillus* abundance were increased linearly with increasing levels of inulin at the end of the 24 hr of incubation but abundance of *E. coli, C. difficile* were decreased linearly with increasing levels of inulin at the end of the 24 hr, although at the 48 hr of incubation the levels of inulin did not affect the abundance of *E. coli*. The *E. coli, C. difficile* abundance were not affected by either 0% starch or 1% starch at any of the levels of inulin at 24 hr of incubation. The abundance of *E. coli* was not affected at 48 hr.

This is consistent with findings by Qing Xia et al. (2012) reported the study was to examine the effect of an infant formula with FOS on select groups of intestinal bacteria in term infants. The formula-fed infants tended to have more total bacteria in their stool samples than the human milk-fed infants. The FOS supplementation at either dose did not significantly increase the *bifidobacterial* or *lactobacilli* populations, or decrease the populations of *C. difficile, E. coli* or *Bacteroides.* Pinna C. et al. (2014) studies *in vitro* effect of some prebiotic substances and 2 dietary

protein levels on the composition and activity of feline fecal microbiota, the bacterial counts after 6 and 24 hr. of incubation *Lactobacillus* and *Bifidobacteria* showed no significant variation attributable to treatment. *C. perfringens* count at 6 hr. were greater ($P<0.05$) than in the control in bottles containing carrot fiber(+1.5 log cells/mL), FOS(+1.9 log cells/mL) and LAC(+1.6 log cells/mL). And after 24 hr the *C. perfringens* count showed no significant variation attributable to treatment.

3.6 Conclusions

The cumulative gas production technique can be used to study the effect of inocula and substrates on *in vitro* fermentation characteristics between inocula and substrate (Williams et al., 2001). The cumulative gas production measured fermentation kinetics and end products, such as total gas production, and VFA were also determined. (Shim et al., 2007). Bauer et al. (2004) reported that fecal inocula can be used for *in vitro* assessment of large intestinal fermentation. The quantification of the species bacteria that are important to animal health and the analysis for the major fermentation products and pH allow for evaluation of these inulin with respect to bacteria and fermentation. However, the result *in vitro* studies cannot be directly extrapolated to *in vivo* studies. However, this *in vitro* study allowed us to evaluate four levels inulin and such *in vitro* studies using fresh fecal samples of animal may be used to test other promising prebiotic ingredients and their combinations simultaneously. The findings of this study may help in designing further evaluate these prebiotics. Overall, the level inulin in these studies had various effects on the species of bacteria and. Future improvement of creep feed for ruminants should be

directed to reduce the abundance of potentially harmful bacteria including *E. coli* and *C. difficile* and increase the abundance of potentially useful bacteria including *Bifidobacterium* and *Lactobacillus*. Therefore, in ruminant animals (cattle, buffalo, goat, sheep, deer), some of microorganisms in the rumen can digest inulin, thereafter the efficiency of inulin in digestive system decreases.

3.7 References

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CHAPTER IV

EFFECT OF INULIN FROM JERUSALEM ARTICHOKE ON THE IMMUNE LEVEL OF YOUNG DAIRY GOATS

4.1 Abstract

This study supplementation the prebiotic effects of dietary inulin from Jerusalem artichoke on young dairy goats. Twenty newborn Saanen kids were sorted by parity of their dams and multiple birth (twin or triplet) and assigned to the five groups (There are five dietary treatments as follows: Treatment 1: Goat milk, Treatment 2: 0% inulin in milk replacer, Treatment 3: 0.1% inulin in milk replacer, Treatment 4: 0.2% inulin in milk replacer and Treatment 5: 0.3% inulin in milk replacer) at birth. Each group in 4 replicates. All kids were fed colostrum for the first 7 days after birth, and then each kid in treatment 3, 4, 5 were supplemented with 0.1, 0.2 and 0.3% inulin on days 8 to 90, respectively.

Fecal score was different ($P<0.05$) in groups. There were differences in fecal score on days 7 to 56 ($P<0.05$), whereas no difference in fecal score on days 70 to 90 ($P>0.05$) was detected in groups. During the current experiment general animal performance data also were collected. Animals fed on goat milk, 0.2 and 0.3% inulin in milk replacer had significantly ($P<0.05$) higher body weight than animals fed on 0 and 0.1% inulin in milk replacer while the control treatment led to intermediate values at 35-90 days of age.Total Red Blood Cell (RBC) and total White Blood Cell (WBC) were significantly ($P<0.01$) in groups. To evaluate whether inulin influence intestinal

morphology, these Length of small intestine and intestinal morphology of goat kids in intestines of experimental in all groups were measured. In jejunum and duodenum length of small intestine showed an overall tendency to be decreased in 0% inulin treated goat kid and to be increased in goat milk, 0.1, 0.2 and 0.3% inulin in milk replacer treated animals when compared to the another group ($P<0.05$). A not similar with villus length in the duodenum, jejunum and ileum. No nutritional effects could be seen for villus width in duodenum, jejunum and ileum or crypt depth.As sterile fecal sample could not be collected from all healthy kids on day 90, sterile fecal were sampled from a subset of healthy kids in each group. In the current study, Goat kids in 0% inulin in milk replacer had significantly higher of fecal suspension for total *Clostridium*, *Escherichia coli*, *Lactobacillus* and *Bifidobacteria* than kids in another treatment. The results of our study suggested that daily dose (0.2%) of inulin might not be enough to observe effects of it. Our data will be useful to determine the dose and timing of inulin supplementation in future studies investigating the effects of inulin on the parameters associated with performance and health status in kids and other young ruminants.

Keywords : inulin, jerusalem artichoke, immune, dairy goats

4.2 Introduction

Currently, the farmers in turn to dairy goats farming are very serious. The goat milks the benefits similar to breast milk. Also, it’s maintained allergies and substitute dairy milk for babies who are allergic to dairy milk. Then, the goat milk is very expensive. But in farming of dairy goats is especially problematic in the young goats

has been a less level of immunity. Which causes such as diarrhea diseases in goat or dairy goats during lactation may be a low immunity level. This is the problem of critical importance to the dairy goats farming are enormous. Because it will decreases production. The research this should help reduce such problems occurred with dairy goats as well.

Jerusalem artichoke (*Helianthus tuberosus* L.) is an herbaceous plant, eaten at its bulb. The bulb of Jerusalem artichoke has important ingredients, i.e. inulin, fructo-oligosaccharide (FOS), protein, fiber, calcium and phosphorus. Jerusalem artichoke can be adapted to growing condition in Thailand and tend to increase planting for both of human diet and also by-products or co-products for animals. Jerusalem artichoke contains 15-20% inulin and FOS. It is considered as prebiotics which have been proposed to improve health by stimulation of beneficial bacteria in the intestine of humans and animals. Inulin is carbohydrate in the form of fructan; it is fiber source which is not digested in digestive system (stomach and intestine) in single stomach animal or human. Therefore, inulin will fall into colon and is benefit to body growth due to an increase in number of health benefit microorganisms, such as *Lactobacillus* and *Bifidobacteria* (Younes et al., 1995; Kaur and Gupta, 2002).

A lot of studies have been conducted to investigate the effects of prebiotics on intestinal microbial populations, digestive and systemic health, immune function and growth performance in non-ruminant. Therefore in ruminant animals (cattle, buffalo, goat, sheep, deer), some of microorganisms in the rumen can digest inulin, thereafter the efficiency of inulin in digestive system decreases. However, there is no information on the effects of supplemental inulin and other prebiotic compounds in the ruminant. The objectives of this study are to investigate the effects of feeding

inulin in milk replacer on fecal score, fecal pH, BW, haematological traits, selected health parameters and the incidence of diarrhea.

4.3 Objective

4.3.1 To study the effect of inulin from Jerusalem artichoke on the heath status of young dairy goats.

4.3.2 To study the effect of inulin from Jerusalem artichoke supplementation on histological in the intestine of dairy goats.

4.4 Materials and methods

4.4.1 Jerusalem artichoke

Jerusalem artichoke were obtained from Phetchabun Research Station, Agro-Ecological System Research and Development Institute, Kasetsart University, Thailand. And Analysis of the Inulin by Modified method based on AOAC method 997.08 (2005) using high temperature gas chromatography (Joye D., and Hoebregs H., 2000). AOAC International from Food and Nutriton Technical Services, Institute of Nutrition, Mahidol University, Salaya, Phuttamonthon, Nakhon Pathom 73170, Thailand.

4.4.2 Animals and experimental design

Twenty newborn crossbred Saanen goats used into a randomly assigned in complete randomized design (CRD) with four replications per treatment. The goat kept in individual pens at 7 day after birth. The goats were divided into five treatment groups. The experiment was conducted for 90 days. There are five dietary treatments as follows: Treatment 1: Goat milk, Treatment 2: 0% inulin in milk replacer,

Treatment 3: 0.1% inulin in milk replacer, Treatment 4: 0.2% inulin in milk replacer and Treatment 5: 0.3% inulin in milk replacer.

4.4.3 Management of feeding

All goat kids were fed colostrum for the first 7 days after birth. And then the goat kid were housed in individual pens. The goats kid were fed a basal diet containing pangola grass (*Digitariaeriantha*) hay and pelleted concentrate supplemented with minerals and vitamins. Feeding management during experimental period of pelleted starter concentrate and hay are presented in Table 4.1 during the suckling period, kids were closely monitored to ensure sufficient sucking. Uniform feeding and management standards were applied in both groups. The diet formulated to meet nutrient requirements for maintenance and 1.5% daily weight gain was offered twice daily at 08.00 and 16.00 h. Animals had free access to water and kept in individual pens.

Table 4.1 Feeding management during experimental period.

Age (days)	Milk (L)	Concentrate / day	Roughage /day
7 - 30	0.7 - 1	-	-
31 - 60	1.5	1.5% body weight	Unlimited
61 - 90	0.5	1.5% body weight	Unlimited

The kids in treatment 3, 4 and 5 were adapted to inulin supplementation by an increased dosage from days 7 to 90. Each kid in was supplemented with 0.1, 0.2 and 0.3 % inulin on treatment 3, 4 and 5 respectively, whereas the kids in treatment 1 was supplemented with Goat milk and treatment 2 was supplemented with milk replacer did not receive inulin. Inulin dissolved mix in milk replacer was to the kids via the suckling.

Table 4.2 Chemical composition of experimental diets.

Item	Concentrate	Jerusalem artichoke	Roughage (Pangola grass hay)
DM	95.76	93.47	94.55
	.....	%DM	.....
CP	15.63	5.47	6.91
Ash	9.04	7.96	7.91
OM	90.96	92.04	92.09
EE	0.08	0.19	0.03
NDF	33.70	11.09	59.04
ADF	25.58	9.57	44.64

DM = dry matter, CP = crude protein, OM = organic matter, EE = ether extract, NDF = neutral detergent fiber, ADF = acid detergent fiber

4.4.4 Sample collection

Health status

Goat kids were monitored daily during the period from birth to weaning by diagnosing diarrhea. Fecal scores of treatment were considered to be diarrhea. Mortality of kids from birth to weaning was recorded. The goat kids were recorded weighed before 08.00 hr. and fecal score every weeks.

Blood collection

Blood samples were collected from the jugular vein after birth (before receiving colostrum) and then catheterized in the afternoon before sampling, when dairy goats were 1 week to 12 weeks old (every week). Blood is determined for total RBC, total WBC count and haematocrit value. Blood was transferred to tubes containing 1.8 mg dipotassium-ethylenediaminetetra acetic acid (EDTA)/ml blood,

then centrifuged at 5,000 xg for 15 min at 4^{o}C; the plasma was partitioned into aliquots and stored at -80 ^{o}C.

Intestinal collection

Slaughtering at 90 day old goats, collected the gastrointestinal tract such as duodenum, jejunum and ileum. Tissue samples were washed in the normal saline solution and fixed in 10% formalin.

4.4.5 Sample analysis

Hematological analysis

Total WBC count was determined in whole blood by means of the chamber counting method developed for counting blood cells. Total WBC in the whole of cross-ruled area of the Thoma counting chamber was counted. Haematocrit value was determined directly by centrifuging blood in a microhaematocrit tube at 10,000xg for 5 min.

Histological analysis

At the end of the experimental, three goat replication from each treatment were sampled the gastrointestinal tract such as duodenum, jejunum and ileum. Tissue samples were washed in the normal saline solution and fixed in 10% formalin. Send samples to The Mahanakorn Veterinary Diagnostic Center. For analysis the length of small intestine and intestinal morphology of goat kids

Fecal microbiota analysis

At the end of 30 day the experimental, three goat replication from each treatment were sampled for microbiological of bacteria by bacteria culture (Total *Clostridium, E. coli, Lactobacillus, Bifidobacteria*). The goats were then dissected under sterile condition. The spread-plate method was used for culture, and bacterial

counts were obtained for the following groups of organisms: Total *Clostridium*, *E. coli*, *Lactobacillus*, *Bifidobacteria.* De Man Rogosa Sharpe agar was used to grow *Lactobacillus* under aerobic conditions for 72 hrs at 30°C. *Clostridium* colonies were counted on lactose egg yolk agar plates after strict anaerobic incubation at 37°C. A geometric dilution series was used for surface plating of the fecal samples; this was accomplished by making serial 10-fold dilutions with 0.15 M NaCl solution from an initial fecal suspension to a final dilution step of 10^9. From each dilution step, 0.1 mL was plated on the dilution agar plates in duplicate. After incubation, the number of bacteria per gram of feces was calculated based on the number of CFU g^{-1}were calculated from statistically viable plates (i.e., plates containing 30-300 colonies), the volume applied to the agar plate and the dilution step using the following formula.

4.4.6 Statistical analysis

Data obtained from the experiment were statistically analyzed according to Completely Randomized Design (CRD) using the PROC GLM procedure (SAS, 1990). Significant differences among treatments were determined using Duncan's News Multiple Range test according to Steel and Torrie (1985).

4.4.7 Experimental location

The experiment was conducted at Suranaree University of Technology's Goat and Sheep Research Farm, the center for Scientific and Technological Equipment, Building 10, Suranaree University of Technology, Nakhon Ratchasima.

Table 4.3 Fecal scoring system.

Fecal	Score
	5 = hard, dry pellets.
	4 = hard, formed
	3 = soft, formed
	2 = soft, unformed
	1 = watery, diarrhea

4.5 Results and discussion

In previous studies with young livestock such as calves (Heinrichs et al., 2003; Hill et al., 2008; Masanetz et al., 2011), lambs (Thayne, 2007; Milewski et al., 2010) and pigs (Davis et al., 2004; Lynch et al., 2007), prebiotics were added to milk replacer or concentrate feed of suckling or weanling animals. However, prebiotics have not been supplemented to animals sucking milk from their dams through oral gavage. Orafti R. GR. contained 93.4% of inulin. Potential adverse side effects, such as diarrhea and flatulence, may occur in animals consuming high levels of fructans or at moderate levels of ingestion in unadapted animals (Propst et al., 2003).

4.5.1 The effects of inulin supplementation on fecal score of kids

Fecal score was different ($P<0.05$) in groups (Table 4.3). There were differences in fecal score on days 7 to 56 ($P<0.05$), whereas no difference in fecal score on days 70 to 90 ($P>0.05$) was detected in groups (Table 4.4).

The result of fecal score in our study was in agreement with the data reported by Propst et al. (2003), who supplemented OF or inulin to adult dogs, and by Hill et al. (2008), who investigated effects of feeding FOS and MOS in dairy calves. Excessive amount of fermentation of fructans by colonic bacteria can lead to increased gas formation, abdominal cramps and loose fecal. This effect is strictly related to dose of fructans (Saavedra, 2005). Verlinden et al. (2006) reported that inulin supplementation lowered fecal score (loose fecal) in adult dogs but the decrease in fecal score had no clinical importance because it remained in an acceptable range and was not associated with diarrhoea. In our study, a lower fecal score would indicate formation of softer fecal and the dose of inulin supplemented to the kids in experimental did not adversely affect fecal score.

Table 4.4 The effects of inulin supplementation on fecal score of kids.

Days	Treatment					P-values
	T1	T2	T3	T4	T5	
7	1.25^{b}	1.25^{b}	2.75^{a}	3.25^{a}	3.50^{a}	0.0001
14	1.75^{cd}	1.50^{d}	2.75^{cb}	3.75^{ab}	4.00^{a}	0.0010
21	1.75^{c}	1.75^{c}	2.25^{c}	3.75^{b}	4.75^{a}	0.0001
28	2.00^{cd}	1.25^{b}	2.50^{c}	3.50^{b}	4.50^{a}	0.0001
35	3.00^{bc}	2.25^{c}	3.25^{b}	4.25^{a}	4.75^{a}	0.0001
42	3.00^{b}	2.50^{b}	4.00^{a}	4.50^{a}	4.75^{a}	0.0002
49	3.50^{bc}	2.75^{c}	3.75^{ab}	4.25^{ab}	4.50^{a}	0.0028
56	3.75^{b}	3.25^{b}	3.75^{b}	3.50^{b}	4.75^{a}	0.0415
63	4.25^{ab}	3.75^{ab}	3.75^{ab}	3.50^{b}	4.75^{a}	0.0867
70	4.25	4.25	4.50	4.75	5.00	0.3458
77	4.75	4.75	4.75	5.00	5.00	0.7362
84	5.00	4.75	5.00	5.00	5.00	0.4380
90	4.75	5.00	5.00	5.00	5.00	0.4380

T1: Goat milk, T2: 0% inulin in milk replacer, T3: 0.1% inulin in milk replacer, T4: 0.2% inulin in milk replacer and T5: 0.3% inulin in milk replacer.

Fecal scoring system: 1 = watery, diarrhea; 2 = soft, unformed; 3 = soft, formed; 4 = hard, formed; and 5 = hard, dry pellets.

4.5.2 The effects of inulin supplementation on body weight of goat kids

During the current experiment general animal performance data also were collected. Animals fed on goat milk, 0.2 and 0.3% inulin in milk replacer had

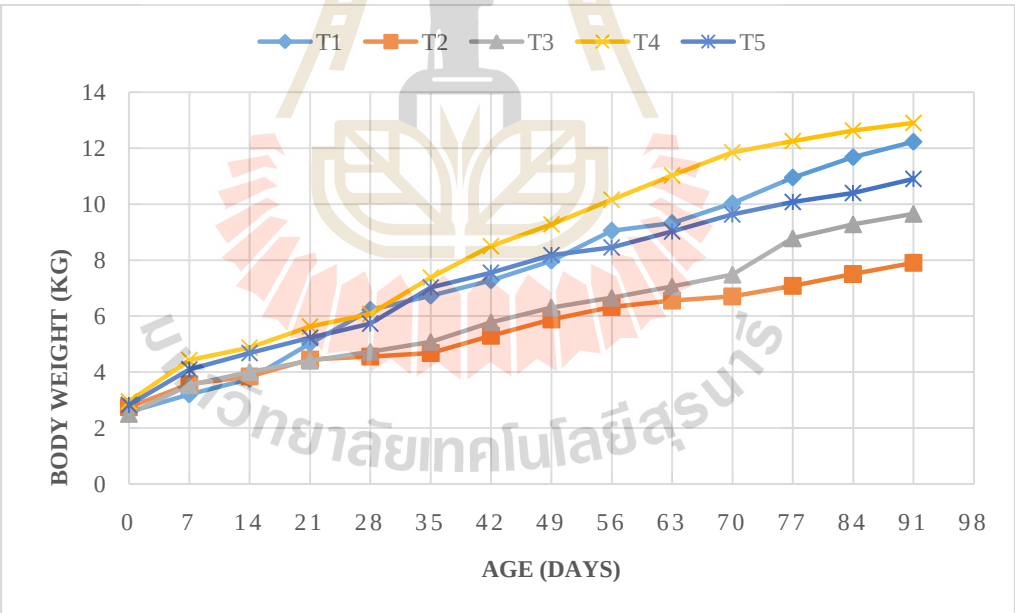
significantly (P<0.05) higher body weight than animals fed on 0 and 0.1% inulin in milk replacer while the control treatment led to intermediate values at 35-90 days of age. (Table 4.5).

Masanetz S. et al. (2010). Studied is aiming to show effects of feeding of either 2% inulin or 2% lactulose in milk replacer on performance and intestinal morphology of male Holstein-Friesian calves. After 20 weeks of feeding inulin led to significantly higher daily weight gains than lactulose while control animals ranged between the experimental feedings.

Kaufhold et al., (2000); Flickinger et al., (2003); Van Loo, (2007). reported that prebiotics are one possible alternative to antimicrobial growth enhancers to improve animal health and performance especially during critical periods such as weaning. Therefore a variety of studies on influences of inulin and oligofructose on animal performance have already been accomplished. Quite a number found no or only little effects, but some reported effects similar to this study with improved daily weight gain and feed efficiency.In contrast, to this results Fleige et al. (2007) found a significantly increased feed consumption and a tendency for improved daily weight gain for calves fed 3% lactulose and a probiotic bacteria strain with their milk replacer.

A satiety effect of prebiotic supplementation can jeopardize the attempt to increase BW gain in livestock (Gaggı `a et al., 2010). Kaufhold et al. (2000) reported that BW only tended to be higher in the calves receiving FOS supplementation lasting for 3 weeks than control calves. Hill et al. (2008) reported no improvement in BW gain of dairy calves fed milk replacer containing FOS or MOS. In contrast, Mul (1997) reported that 2 to 5g/kg OF added to milk replacer diets of calves improved

BW gain. Xu et al. (2002) reported that 4 or 6g/kg FOS added to the diet improved BW gain in growing pigs. The results of prebiotic supplementation on the growth performance (BW, BW gain and feed efficiency) of livestock are often contradictory and mostly affected by the compound chosen, the dietary supplementation level, duration of use and the environmental and stress status of the animals (Gaggı `a et al.,2010).The low or absent effect on grow the performance in animals supplemented with prebiotics can be due to enough prebiotic compounds in the basal diet (Flickinger et al ., 2003a; Gaggı `a et al., 2010). In the current study, the absent effect of inulin on BW in suckling kids may be due to high content of prebiotics in goat milk (25 to 30 mg/100ml; Mart ´ne ´z-Fe ´rez et al., 2005) and the period of inulin supplementation(25days) being too short.



T1: Goat milk, T2: 0% inulin in milk replacer, T3: 0.1% inulin in milk replacer, T4: 0.2% inulin in milk replacer and T5: 0.3% inulin in milk replacer.

Figure 4.2 Show the effects of inulin supplementation on body weight of goat kids

Table 4.5 The effects of inulin supplementation on body weight of goat kids.

Age (days)	Treatment (n=4)					SEM	P-values	Contrast	Significant of group difference		
	T1	T2	T3	T4	T5			T2, T3, T4, T5	T1 vs T2, T3, T4, T5	T1 vs T3, T4, T5	T2 vs T3, T4, T5
Birth	2.575±0.21	2.750±0.66	2.500±0.26	2.950±0.51	2.825±0.66	0.0551	0.7054	ns, ns, ns	0.5235	0.5320	0.9772
7	3.200±0.67	3.575±0.87	3.525±0.37	4.425±1.11	4.100±1.10	0.1694	0.3257	ns, ns, ns	0.1672	0.1249	0.3933
14	3.750±0.87	3.850±0.26	4.000±0.72	4.875±1.32	4.675±1.26	0.2089	0.3859	ns, ns, ns	0.2843	0.1897	0.2508
21	5.025±1.11	4.450±1.20	4.400±0.94	5.625±1.98	5.225±1.33	0.4134	0.6756	ns, ns, ns	0.8971	0.9417	0.4324
28	6.225±1.30	4.550±0.83	4.725±1.09	6.075±1.72	5.725±1.72	0.4251	0.3275	ns, ns, ns	0.2338	0.3822	0.2473
35	$6.725±1.64^{ab}$	$4.675±0.67^{c}$	$5.075±0.89^{bc}$	$7.375±1.43^{a}$	$7.025±0.83^{ab}$	0.2993	0.0146	***, *, *	0.3046	0.7317	0.0158
42	$7.275±1.81^{ab}$	$5.300±0.87^{b}$	$5.775±1.43^{b}$	$8.500±1.47^{a}$	$7.550±1.67^{ab}$	0.4923	0.0454	**, ns, *	0.5605	1.0000	0.0358
49	$7.975±2.01^{ab}$	$5.875±1.34^{b}$	$6.300±1.56^{b}$	$9.275±1.55^{a}$	$8.175±1.70^{ab}$	0.6078	0.0576	**, ns, *	0.5464	0.9519	0.0487
56	$9.050±2.18^{ab}$	$6.325±1.36^{c}$	$6.650±1.53^{bc}$	$10.150±1.59^{a}$	$8.450±1.59^{abc}$	0.6247	0.0262	**, ns, **	0.2349	0.5216	0.0467
63	$9.325±2.65^{ab}$	$6.550±1.54^{b}$	$7.050±1.32^{b}$	$11.025±1.44^{a}$	$9.025±1.34^{ab}$	0.6715	0.0150	**, ns, **	0.3611	0.7746	0.0254
70	$10.025±2.72^{ab}$	$6.700±1.69^{c}$	$7.475±1.37^{bc}$	$11.850±1.36^{a}$	$9.625±1.48^{ab}$	0.7217	0.0073	***, ns, ns	0.2854	0.7227	0.0123
77	$10.950±2.42^{ab}$	$7.075±1.83^{c}$	$8.775±1.39^{bc}$	$12.250±1.36^{a}$	$10.075±1.44^{ab}$	0.6750	0.0075	***, **, *	0.1682	0.5695	0.0050
84	$11.675±2.28^{ab}$	$7.500±1.89^{c}$	$9.275±1.38^{bc}$	$12.625±1.41^{a}$	$10.400±1.41^{ab}$	0.6548	0.0061	***, **, *	0.0915	0.3724	0.0048
91	$12.225±2.14^{ab}$	$7.900±1.86^{c}$	$9.650±1.40^{bc}$	$12.900±1.43^{a}$	$10.900±1.23^{ab}$	0.6055	0.0045	***, **, *	0.0581	0.2756	0.0038

T1: Goat milk, T2: 0% inulin in milk replacer, T3: 0.1% inulin in milk replacer, T4: 0.2% inulin in milk replacer and T5: 0.3% inulin in milk replacer.

ns = not significant, only significant (***P<0.01, **P<0.05, *P<0.1)

4.5.3 The effects of inulin supplementation on haematological traits of goat kids

Blood samples were collected from a subset of healthy kids in each group. WBC on 0% inulin was higher when compared with the standard (Table 4.7). Swanson et al. (2002a) reported that there were no significant changes in the total WBC count and neutrophil concentration in adult dogs supplemented with FOS (2g/day), MOS (2g/day) or FOS (2g/day) plus MOS (2g/day). Davis et al. (2004) observed the increase in blood lymphocyte concentration and the decrease in blood neutrophil concentration when pigs were fed the diets supplemented with 0.3% MOS. The increase in blood lymphocyte concentration maybe useful because of the increased level of protection from re-infection of a pathogen, whereas the decrease in blood neutrophil concentration may be a negative outcome of feeding prebiotics as neutrophils play a key role in the first line of defense against infectious organisms (Swanson et al., 2002b; Davis et al., 2004). Verlinden et al. (2006) reported that 3% inulin added to the diet did not change blood neutrophil, lymphocyte and monocyte concentrations and haematocrit value in dogs. Masanetz et al. (2011) reported no change in the total WBC count, blood neutrophil, lymphocyte and monocyte concentrations of calves fed the diet containing 2% inulin.The results of Verlinden et al. (2006) and Masanetz et al. (2011) were in agreement with those of our study. Studies where an immune challenge is presented may be conducted to determine whether changes in the concentrations of lymphocyte and neutrophil from blood immune characteristics are useful or harmful. Different haematological results on the.effects of inulin or other prebiotic compounds may be obtained for kids facing an immune challenge.

Table 4.6 The effects of inulin supplementation on total RBC (10^6 cells/mm^3) of goat kids.

Days	Treatment					SEM	P-values	Contrast	Significant of group difference		
	T1	T2	T3	T4	T5			T2, T3, T4, T5	T1 vs T2, T3, T4, T5	T1 vs T3, T4, T5	T2 vs T3, T4, T5
0	7.99±0.64	8.37±0.96	8.89±4.75	9.94±2.21	9.46±1.73	1.4210	0.8103	ns, ns, ns	0.4160	0.3371	0.4783
7	7.06±1.24	8.17±1.71	8.88±1.14	6.16±3.00	7.08±0.74	0.6835	0.2564	ns, ns, *	0.6055	0.7581	0.4428
14	9.401±2.40	9.48±0.73	10.24±2.10	7.48±3.29	8.96±1.30	1.0377	0.4856	ns, ns, ns	0.7640	0.6852	0.6453
21	11.56±1.28^{a}	9.29±2.17^{ab}	11.62±2.10^{a}	10.31±1.06^{ab}	7.84±0.98^{b}	0.5741	0.0213	ns, ***, ns	0.0636	0.0972	0.5057
28	12.85±0.80	10.51±1.32	10.93±2.56	12.84±1.39	12.64±0.48	0.4958	0.1024	**, ns, ns	0.1982	0.4186	0.0786
35	18.66±7.09^{a}	11.39±1.38^{b}	12.60±3.60^{ab}	15.16±3.95^{ab}	13.42±0.51^{ab}	3.6194	0.1514	ns, ns, ns	0.0269	0.0507	0.3311
42	14.88±2.24^{a}	12.25±1.57^{b}	12.23±0.89^{b}	14.12±0.85^{ab}	14.02±0.95^{ab}	0.4418	0.0582	**, ns, ns	0.0449	0.1016	0.1555
49	13.68±1.22^{ab}	12.23±1.39^{b}	13.35±0.66^{ab}	14.22±1.14^{a}	13.36±1.16^{ab}	0.2905	0.2194	ns, ns, ns	0.5514	0.9578	0.0488
56	11.32±1.14^{b}	10.99±1.18^{b}	12.15±1.63^{ab}	12.24±1.21^{ab}	13.95±1.20^{a}	0.3688	0.0439	***, ns, ns	0.1797	0.0681	0.0290
63	13.97±1.67^{a}	10.32±1.24^{b}	13.68±1.08^{a}	13.36±1.36^{a}	12.87±1.63^{a}	0.4474	0.0162	**, **, ns	0.0940	0.4265	0.0023
70	12.55±1.41^{a}	8.16±0.73^{b}	12.53±1.77^{a}	11.47±1.11^{a}	13.36±1.42^{a}	0.3986	0.0005	***, *, **	0.1392	0.9068	0.0001
77	13.72±1.61^{a}	8.23±0.87^{b}	12.10±1.71^{a}	12.86±1.83^{a}	13.24±1.27^{a}	0.5030	0.0008	***, **, ns	0.0235	0.2713	0.0001
84	14.07±1.25^{a}	8.43±0.96^{b}	12.48±1.15^{a}	13.01±0.98^{a}	13.97±1.23^{a}	0.2816	0.0001	***, **, ns	0.0045	0.1787	0.0001
91	12.46±1.84^{a}	7.35±1.21^{b}	12.53±1.68^{a}	14.08±1.11^{a}	14.09±1.21^{a}	0.4645	0.0001	***, ***, ns	0.5873	0.2024	0.0001

T1: Goat milk, T2: 0% inulin in milk replacer, T3: 0.1% inulin in milk replacer, T4: 0.2% inulin in milk replacer and T5: 0.3% inulin in milk replacer.

ns = not significant, only significant (***P<0.01, **P<0.05, *P<0.1)

4.7 The effects of inulin supplementation on Total WBC (10^3cells/mm^3) of goat kids.

Days	Treatment					SEM	P-values	Contrast	Significant of group difference		
	T1	T2	T3	T4	T5			T2, T3, T4, T5	T1 vs T2, T3, T4, T5	T1 vs T3, T4, T5	T2 vsT3, T4, T5
0	4.30±1.13	3.01±0.71	4.91±1.79	4.80±2.57	3.73±1.20	0.5826	0.4607	ns, *,ns	0.8397	0.8487	0.1364
7	6.29±2.32	5.81±2.16	7.19±2.07	6.65±0.64	7.63±3.55	1.2221	0.8217	ns, ns, ns	0.6927	0.5325	0.3353
14	8.54±3.67^{ab}	4.13±1.38^{b}	6.06±2.15^{ab}	8.29±2.55^{ab}	9.87±3.53^{a}	1.7446	0.0742	***, ns, ns	0.3658	0.7749	0.0272
21	4.89±2.52^{c}	12.24±1.38^{a}	7.37±1.13^{bc}	7.95±1.86^{b}	9.98±2.18^{ab}	0.7941	0.0008	ns, ***, ***	0.0007	0.0053	0.0032
28	8.17±1.40	8.91±2.06	8.13±2.04	10.44±2.42	10.86±3.12	1.1624	0.3309	ns, ns, ns	0.2861	0.2335	0.5056
35	6.18±1.28^{b}	9.93±2.28^{ab}	10.39±3.80^{a}	10.97±2.57^{a}	10.02±2.21^{ab}	1.4662	0.1180	ns, ns, ns	0.0110	0.0111	0.7246
42	8.56±1.77	9.93±2.01	11.95±3.06	11.36±3.11	9.64±2.06	1.3630	0.3436	ns, ns, ns	0.1386	0.1100	0.4725
49	9.45±1.50	10.34±2.40	12.63±2.30	10.37±1.90	9.36±1.95	0.9256	0.2078	ns, ns, ns	0.2964	0.2715	0.7116
56	8.24±1.94^{b}	11.78±2.20^{a}	11.25±2.01^{ab}	10.61±2.19^{ab}	9.36±1.72^{ab}	0.9110	0.1406	ns, ns, ns	0.0421	0.0830	0.2572
63	7.29±1.02^{c}	12.79±1.56^{a}	11.80±1.18^{a}	9.86±1.04^{b}	8.89±0.97^{bc}	0.3089	0.0001	***, ns, ns	0.0001	0.0007	0.0016
70	9.51±1.01^{c}	13.89±1.37^{a}	11.64±1.58^{b}	9.24±1.24^{c}	9.53±1.08^{c}	0.3620	0.0004	***, *, ns	0.0444	0.4109	0.0001
77	8.91±1.07^{b}	13.95±1.24^{a}	12.46±1.80^{a}	8.94±1.10^{b}	9.94±0.99^{b}	0.3635	0.0001	***, *, **	0.0041	0.0538	0.0003
84	8.18±1.34^{b}	14.42±1.25^{a}	10.31±1.21^{b}	9.37±1.57^{b}	9.74±1.50^{b}	0.4272	0.0002	***, ***, ns	0.0026	0.0589	0.0001
91	9.15±1.27^{b}	14.18±1.21^{a}	10.79±1.33^{b}	9.94±1.57^{b}	10.54±1.73^{b}	0.4620	0.0019	***, **, ns	0.0150	0.1478	0.0004

T1: Goat milk, T2: 0% inulin in milk replacer, T3: 0.1% inulin in milk replacer, T4: 0.2% inulin in milk replacer and T5: 0.3% inulin in milk replacer.

ns = not significant, only significant (***P<0.01, **P<0.05, *P<0.1)

Table 4.8 The effects of inulin supplementation on haematocrit (%) of goat kids.

Days	Treatment					SEM	P-values	Contrast	Significant of group difference		
	T1	T2	T3	T4	T5			T2, T3, T4, T5	T1 vs T2, T3, T4, T5	T1 vs T3, T4, T5	T2 vs T3, T4, T5
0	39.50±3.70	38.25±4.27	44.50±8.58	38.75±8.85	38.75±4.99	9.3355	0.6428	ns, ns, ns	0.8783	0.7588	0.5269
7	31.50±3.00	31.50±1.91	30.50±5.26	29.25±6.40	30.25±2.50	3.9131	0.9321	ns, ns, ns	0.6374	0.5439	0.5439
14	31.00±2.16	32.00±5.72	35.00±4.24	33.25±3.86	31.50±3.11	3.5740	0.6392	ns, ns, ns	0.3996	0.3451	0.5961
21	29.50±2.38	32.50±1.29	34.00±6.06	31.75±6.65	31.25±1.71	4.0771	0.6689	ns, ns, ns	0.2471	0.2684	0.9470
28	29.75±2.06	30.00±2.16	30.50±5.74	32.75±6.13	31.50±1.73	3.6895	0.8276	ns, ns, ns	0.5362	0.4465	0.5099
35	31.00±2.58	29.75±1.71	31.75±5.56	34.25±6.13	32.00±2.94	3.8796	0.6507	ns, ns, ns	0.6929	0.4989	0.2440
42	31.50±4.65	28.75±1.50	33.33±5.13	31.25±6.65	32.50±5.20	5.4011	0.7653	ns, ns, ns	0.9882	0.7691	0.2299
49	33.00±6.38	28.00±2.71	31.25±3.20	29.75±3.86	29.50±6.56	5.1951	0.6546	ns, ns, ns	0.2295	0.3248	0.4483
56	33.25±4.19	29.75±5.91	34.25±2.75	31.75±2.63	32.50±8.23	6.0224	0.7856	ns, ns, ns	0.6881	0.8913	0.3198
63	35.75±5.56^{ab}	27.25±5.97^{b}	37.25±4.19^{a}	34.50±3.00^{ab}	33.50±7.00^{ab}	6.3541	0.1345	ns, ns, ns	0.3923	0.8314	0.0224
70	38.25±4.65^{a}	28.25±5.56^{b}	36.50±3.42^{a}	39.50±1.00^{a}	36.00±5.16^{a}	4.1069	0.0177	**, **, ns	0.2032	0.7162	0.0023
77	41.25±6.18^{a}	29.00±6.63^{b}	37.50±6.03^{a}	41.50±2.52^{a}	37.25±0.96^{a}	5.6274	0.0200	**, **, ns	0.0987	0.4016	0.0042
84	41.75±5.32^{a}	27.00±5.35^{b}	37.75±2.87^{a}	40.25±3.86^{a}	38.00±1.41^{a}	3.6709	0.0010	***, ***, ns	0.0182	0.2073	0.0002
91	42.25±5.56^{a}	27.00±6.16^{b}	40.25±2.06^{a}	44.00±4.83^{a}	38.50±0.58^{a}	4.3305	0.0006	***, ***, ns	0.0693	0.6074	0.0001

T1: Goat milk, T2: 0% inulin in milk replacer, T3: 0.1% inulin in milk replacer, T4: 0.2% inulin in milk replacer and T5: 0.3% inulin in milk replacer.

ns = not significant, only significant (***P<0.01, **P<0.05, *P<0.1)

4.5.4 The effects of inulin supplementation on rumen fluid and digest pH of goat kids

Table 4.9 The effects of inulin supplementation on rumen fluid and digest pH of goat kids.

Item	Treatment					SEM	P-values	Contrasts	Significant of group difference		
	T1	T2	T3	T4	T5			T2, T3, T4, T5	T1 vs T2, T3, T4, T5	T1 vs T3, T4, T5	T2 vs T3, T4, T5
Rumen fluid	7.22±0.20^{b}	7.70±0.09^{a}	6.92±0.15^{b}	6.99±0.37^{b}	6.97±0.06^{b}	0.0093	0.0004	***, ***, *	0.5512	0.0465	0.0001
Digest pH											
- Jejunum	6.59±0.25^{c}	6.90±0.10^{b}	6.32±0.06^{d}	7.19±0.10^{a}	6.52±0.11^{cd}	0.0045	0.0001	ns, ns, ***	0.0880	0.2923	0.0164
- Ileum	7.59±0.37^{b}	7.17±0.11^{c}	6.43±0.08^{d}	7.99±0.12^{a}	7.73±0.12^{ab}	0.0084	0.0001	***, **, ***	0.0270	0.0777	0.0746

T1: Goat milk, T2: 0% inulin in milk replacer, T3: 0.1% inulin in milk replacer, T4: 0.2% inulin in milk replacer and T5: 0.3% inulin in milk replacer.

ns = not significant, only significant (***P<0.01, **P<0.05, *P<0.1)

4.5.5 The effects of inulin supplementation on Length of small intestine and intestinal morphology of goat kids

To evaluate whether inulin influence intestinal morphology, these Length of small intestine and intestinal morphology of goat kids in intestines of experimental in all groups were measured (Table 4.10). In jejunum and duodenum length of small intestine showed an overall tendency to be decreased in 0% inulin treated goat kid and to be increased in goat milk, 0.1, 0.2 and 0.3%inulin in milk replacer treated animals when compared to the another group ($P<0.05$). A not similar with villus length in the duodenum, jejunum and ileum. No nutritional effects could be seen for villus width in duodenum, jejunum and ileum or crypt depth.

Masanetz S. et al., (2010). Reported, ingestion of milk replacer was reduced in lactulose treated animals. Additionally differences of villus height in jejunum ($P<0.07$) and ileum ($P<0.03$) could be found with an increase for lactulose treated animals and a decrease for inulin treated animals. In ileum the density of proliferative epithelial cells tended to be lower in inulin treated and higher in lactulose treated animals ($P<0.08$). Both prebiotics can affect performance and intestinal morphology of calves and may as such affect animal health. But effects differ between substances.

Wu et al., (1996); Awad et al., (2008) Reported that interestingly a significantly higher daily weight gain or an improved feed conversion ratio as was found in the inulin group is commonly associated with a better intestinal nutrient absorption because of longer villi, while in this study small intestinal villi were shortened after inulin feeding. A similar decrease of villus length after addition of inulin to the feed has already been reported in weaning piglets (Pierce et al., 2005). But also increases of villus length in rats after feeding of soluble chicory extract (1%

to 5%) or purified inulin (5%) (Kim, 2002) were found. Supporting the observed shortening of villi a decrease of MIB1-positive and hence considered proliferating cells was found in ileum of calves fed inulin. A comparable lowering of cell proliferation has been reported before in rats fed inulin and OF at a concentration of 10% of the diet (Femia et al., 2002). Concluding it may have to be considered that shorter villi and a decreased proliferation rate may reduce the amount of energy necessary for maintenance of gut architecture thereby providing more energy for growth and fattening. Although lactulose has also been considered as a prebiotic only comparably little data on animal performance has been available up until now. In contrast, to this results Fleige et al. (2007) found comparable to these findings with inulin they also found a decrease of villus length in ileum of said calves. Directly opposed to these effects lactulose treatment in this study led to lower feed intakes and daily weight gains and simultaneously increased villus heights in the small intestine compared to the control group. Possibly the addition of *Enterococcus faecium* to the milk replacer in the study of Fleige et al. (2007) had a modulating influence on lactulose derived effects leading to different regulations of appetite and villus length. In accordance to the longer villi lactulose also tended to increase the number of proliferative cells in ileum. Similar to this finding other dietary fibers such as highly fermentable guar gum and pectin are also known to increase the proliferation zone in the caecum crypts of rats (Brunsgaard and Eggum, 1995). Interestingly the notable decrease of nutrient ingestion (10%) of lactulose fed calves was not enough to antagonize the increase of villus length or proliferation rates as may be expected with respect to studies on fasted animals (Clarke, 1975). But, on the other hand, no reduction in villus height was found in pigs fed with a low-energy diet compared to a

diet with twice the energy content (Claus et al., 2006) which would be more similar to the decrease in energy uptake in the lactulose group than starved animals. An expected improvement of animal performance due to a better intestinal morphology (Wu et al., 1996; Awad et al., 2008) may be overruled by the less nutrient ingestion observed in the lactulose group. But similar to inulin lactulose also led to a decrease in goblet cell density in villus tips in ileum. The differences in effects on animal performance and mucosal architecture between the treatment groups are not easily explained by differences in nutrient consumption alone but could be based on partly different fermentation properties of the two prebiotics. It has been found that pre-caecal fermentability of lactulose in pigs was lower than that of inulin (Branner et al., 2004) maybe leading to different sites of action in the calves' intestines. Additionally both substances differed in stimulatory effects on beneficial bacterial subpopulations and fermentation product profiles (Rycroft et al., 2001). In conclusion this study has shown regulative effects of level of inulin commonly used inulin on intestinal villus length supporting observations already made before in other animals. But the differences in the effects of the level of inulin on animal performance and intestinal morphology observed in this study raise the question whether inulin and their health promoting actions can be generalized. In conclusion the usage of prebiotics would have to be fine tuned for particular purposes raising the need for additional research study.

Table 4.10 The effects of inulin supplementation on Length of small intestine and intestinal morphology of goat kids

Item	Treatment					P-values	Contrast	Significant of group difference		
	T1	T2	T3	T4	T5		T2, T3, T4, T5	T1 vs T2, T3, T4, T5	T1 vs T3, T4, T5	T2 vs T3, T4, T5
Length of small intestine (cm)										
Duodenum	100.00±20.99^{a}	54.75±9.54^{c}	87.50±8.96^{ab}	69.75±17.11^{bc}	104.25±5.32^{a}	0.0006	***, ns, ***	0.0151	0.1245	0.0009
Jejunum	361.50±32.05^{b}	228.25±15.73^{c}	303.25±19.03^{bc}	518.00±141.44^{a}	292.00±34.98^{bc}	0.0003	**, ***, ***	0.5001	0.8094	0.0023
Ileum	361.50±70.35^{cd}	641.50±43.94^{a}	414.75±60.15^{c}	319.50±26.30^{d}	508.00±37.98^{b}	0.0001	***, ***, ns	0.0014	0.0901	0.0001
Intestinal morphology (µm)										
DVL	649.42±102.85^{a}	541.94±68.18^{ab}	471.43±97.06^{bc}	451.96±66.61^{bc}	376.31±50.39^{c}	0.0007	***, ns, ns	0.0001	0.0001	0.0123
DCL	161.90±8.84^{bc}	290.09±33.17^{a}	206.23±70.09^{b}	135.53±52.19^{c}	319.06±28.20^{a}	0.0001	ns, ***, ns	0.0003	0.0014	0.0643
JVL	223.88±51.56^{b}	472.85±160.27^{a}	273.80±30.87^{b}	191.41±32.06^{b}	515.55±131.84^{a}	0.0001	ns, ***, ns	0.0098	0.0545	0.0090
JCL	177.89±12.55^{b}	135.44±32.28^{c}	185.01±17.72^{b}	101.94±19.22^{d}	298.78±19.27^{a}	0.0001	***, ***, ***	0.4027	0.0266	0.0001
IVL	359.05±55.70^{a}	175.62±32.43^{b}	170.53±66.66^{b}	143.77±38.43^{b}	125.03±14.53^{b}	0.0001	*, ns, ns	0.0001	0.0001	0.0001
ICL	224.93±31.40^{a}	182.23±29.10^{b}	145.19±21.93^{c}	87.41±11.79^{d}	107.54±11.54^{d}	0.0001	***, ns, ns	0.0001	0.0001	0.0001

T1: Goat milk, T2: 0% inulin in milk replacer, T3: 0.1% inulin in milk replacer, T4: 0.2% inulin in milk replacer and T5: 0.3% inulin in milk replacer.

Duodenum Villuos length (DVL), Duodenum Crypt length (DCL), Jejunum Villuos length (JVL), Jejunum Crypt length (JCL), Ileum Villuos length (IVL), Ileum Crypt length (ICL)

ns = not significant, only significant (***P<0.01, **P<0.05, *P<0.1)

Table 4.11 The effects of inulin supplementation of the fecal bacterial populations of goat kids

Item	Treatment					SEM	P-values	Contrast	Significant of group difference		
	T1	T2	T3	T4	T5			T2, T3, T4, T5	T1 vs T2, T3, T4 ,T5	T1 vs T3, T4, T5	T2 vs T3, T4, T5
Digest bacterial populations in the fecal (cfu log_{10}/g fresh fecal)											
Total Clostridium	0.86±0.02^{c}	1.18±0.02^{a}	1.16±0.03^{a}	0.92±0.03^{b}	0.89±0.04^{bc}	0.0002	0.0001	***, ns, ***	0.0001	0.0001	0.0001
Escherichia coli	1.98±0.03^{c}	2.55±0.03^{a}	2.32±0.03^{b}	0.67±0.03^{d}	0.65±0.03^{d}	0.0002	0.0001	***, ***, ***	0.0001	0.0001	0.0001
Lactobacillus	6.91±0.03^{a}	5.02±0.04^{e}	5.23±0.03^{d}	6.49±0.02^{c}	6.79±0.03^{b}	0.0002	0.0001	***, **, ***	0.0001	0.0001	0.0001
Bifidobacteria	6.93±0.02^{a}	5.21±0.02^{e}	6.20±0.04^{c}	6.52±0.01^{c}	6.89±0.02^{b}	0.0002	0.0001	***, ***, ***	0.0001	0.0001	0.0001

T1: Goat milk, T2: 0% inulin in milk replacer, T3: 0.1% inulin in milk replacer, T4: 0.2% inulin in milk replacer and T5: 0.3% inulin in milk replacer.

ns = not significant, only significant (***P<0.01, **P<0.05, *P<0.1)

4.5.6 The effects of inulin supplementation of fecal bacteria in goat kids

As sterile fecal sample could not be collected from all healthy kids on day 90, sterile fecal were sampled from a subset of healthy kids in each group. In the current study, Goat kids in 0% inulin in milk replacer had significantly higher CFU of fecal suspension for total clostridium, *E. coli*, *Lactobacillus* and *Bifidobacteria* than kids in another treatment (table 4.11).

Barry et al. (2009) reported similar results for concentration of fecal *E.coli* in adult dogs when 0.2% or 0.4% inulin was added to the diet. Lynch et al. (2007) observed that inulin supplementation did not change *E. coli* concentration in the colon of pigs. Bunce et al. (1995) reported that 3 or 7g/day OF supplemented to calves did not significantly decrease fecal concentrations of total *Clostridium* and *E. coli*. Swanson et al. (2002a) reported no significant differences in fecal bacterial populations of adult dogs among groups with FOS (2g/day), MOS (2g/day), FOS (2g/day) plus MOS (2g/day) and without prebiotics.

4.6 Conclusions

This study demonstrated the beneficial effect of inulin on growth, health status, haematological traits, rumen fluid and intestinal morphology in goat kid. In addition, the commended levels of dietary supplementation with Jerusalem artichoke had superior with 0.2% of inulin in milk replacer.

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CHAPTER V

OVERALL CONCLUSION AND IMPLICATION

5.1 Conclusion

The purposes of the present study were to investigate the potential of inulin from plants, Chicory (*Cichoriumintybus* L.) and Jerusalem artichoke (*Helianthus tuberosus* L.) as an alternative prebiotic have contains 15-20% inulin for young ruminants. The objectives of this study are to investigate the effects of feeding inulin in milk replacer on fecal score, body weight, haematological traits, selected health parameters and the incidence of diarrhea.The present studies were successful.

Experiment I, Although several studies have presented the inulin effects of animal feed and using *in vitro* fermentation of rumen fluid samples, no study has been reported that fermentation used *in vitro* culture of calve fecal samples.The objective of this study was to the effect of inulin from chicory level on the abundance of bacteria and their fermentation using *in vitro* of calve fecal samples. Two levels of starch and 4 levels of inulin from chicory were a completely randomized design with a 2 x 4 factorial arrangement of treatments. Factors consisted of starch levels (0% starch and 1% starch respectively) and inulin from chicory levels (0%, 1%, 2% and 4%, respectively). Fresh fecal samples were collected from 3 Jersey calves after feeding the milk (approximately 32 days after birth). In the culture, sterile medium + fecal slurry +Treatment these culture tubes were carried out in triplicate and incubated 37 ^{o}C for 24, 48 hr in a shaking incubator. Total bacteria, *Bifidobacterium*,

Clostridium difficile, *E. coli*, *Lactobacillus* and *Salmonell*a were quantified using respective specific real-time PCR assays. Volatile fatty acid (VFA) concentrations were analysis using gas chromatography. The pH values were recorded using a pH meter. Gas pressure was measured using a manometer. Inulin, at present mainly extracted from chicory used in this study was increased the total gas production of fecal fermentation with inulin at the end of the 24 hr was increased linearly when increasing percent of inulin which is simultaneously reflected by the large increase in gas production at high percent of inulin and gas production when the time of incubation increase (from 0 to 24 hr). In contrast, the pH values of the fecal fermentation at 24 hr was decreased linearly ($P<0.001$) with increasing percent of inulin. The VFA concentrations at the end of the 24 and 48 hr of incubation were influence the levels of inulin. The proportion of A/P ratio was significantly at 24 and 48 hr of incubation. The addition of starch (0% starch and 1% starch) have influence acetate, butyrate and A/P ratio ($P<0.001$) at the end of the 24 hr but at the 48 hr did not influence butyrate, valerate and iso-butyrate. Nevertheless, in this study compare with control (0%inulin) significant reduced acetic acid and increased propionic and butyric acid proportions. The addition of 0% starch or 1% starch did not differ the abundance of *E. coli*, *C. difficile* at 24 hr and *E. coli* at 48 hr incubation. The abundance of Total bacteria, *Bifidobacterium* and *Lactobacillus* abundance were increased linearly with increasing levels of inulin but abundance of *E. coli*, *C. difficile* were decreased linearly with increasing levels of inulin. In general, the reduction of intestinal pH is a positive outcome, as a more acidic environment might protect against undesirable and pathogenic bacteria. *In vitro* fermentation and animal studies have demonstrated that supplementing the diet with inulin decreased the pH. Inulin

did not suppress *E. coli* and *C. difficile* which are harmful microbes cause of diarrhea in the ruminant animals. But supported *bifidobacterial* and *lactobacillus* abundance comparable with high levels of inulin. However, some of microorganisms in the rumen can digestion inulin, thereafter the efficiency of inulin in digestive system decreases. But the digestive system of the young goat, and other young ruminants is very similar to that of the pig and human (explaining the term 'preruminant').

Experiment II, this study supplementation the prebiotic effects of dietary inulin from Jerusalem artichoke on young dairy goats. Twenty newborn Saanen kids were sorted by parity of their dams and multiple birth (twin or triplet) and assigned to the five groups (There are five dietary treatments as follows: Treatment 1: Goat milk, Treatment 2: 0% inulin in milk replacer, Treatment 3: 0.1% inulin in milk replacer, Treatment 4: 0.2% inulin in milk replacer and Treatment 5: 0.3% inulin in milk replacer) at birth. Each group in 4 replicates. All kids were fed colostrum for the first 7 days after birth, and then each kid in treatment 3, 4, 5 were supplemented with 0.1, 0.2 and 0.3% inulin on days 8 to 90, respectively.

Fecal score was different ($P<0.05$) in groups. There were differences in fecal score on days 7 to 56 ($P <0.05$), whereas no difference in fecal score on days 70 to 90 ($P>0.05$) was detected in groups. During the current experiment general animal performance data also were collected. Animals fed on goat milk, 0.2 and 0.3% inulin in milk replacer had significantly ($P<0.05$) higher body weight than animals fed on 0 and 0.1% inulin in milk replacer while the control treatment led to intermediate values at 35-90 days of age.Total Red Blood Cell (RBC), total White Blood Cell (WBC) and haematocrit value were similar for all treatment. There were no differences ($P >0.05$) in total RBC, total WBC and haematocrit value in groups. To evaluate whether inulin

influence intestinal morphology, these Length of small intestine and intestinal morphology of goat kids in intestines of experimental in all groups were measured. The jejunum and duodenum length of small intestine showed an overall tendency to be decreased in 0% inulin treated goat kids and to be increased in goat milk, 0.1, 0.2 and 0.3% inulin in milk replacer treated animals when compared to the another group ($P<0.05$). A not similar with villus length in the duodenum, jejunum and ileum. No nutritional effects could be seen for villus width in duodenum, jejunum and ileum or crypt depth. As sterile fecal sample could not be collected from all healthy kids on day 90, sterile feces were sampled from a subset of healthy kids in each group. In the current study, Goat kids in 0% inulin in milk replacer had significantly higher CFU of fecal suspension for total clostridium, *E. coli*, *Lactobacillus* and *Bifidobacteria* than kids in another treatment. The results of our study suggested that daily level 0.2% inulin in milk replacer for goat kid.

5.2 Implication

The present study suggests that the study of inulin supplemented to young dairy goats did adversely effect fecal score. The results of our study suggested that daily dose (0.2%) of inulin might not be enough to observe effects of it. Our data will be useful to determine the dose and timing of inulin supplementation in future studies investigating the effects of inulin on the parameters associated with performance and meat quality in young dairy goats and other young ruminants.

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